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ASCOSCHÖNGASTIA BUSHLANDI, A NEW SPECIES OF CHIGGER MITE FROM DUTCH NEW GUINEA[1]

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Among the considerable numbers of trombiculid mites taken on various hosts during an extensive epidemic of tsutsugamushi disease in American Forces on Owi Island, Dutch New Guinea, (Kohls et al, 1946 "Area D"), it is strange that the present new species did not turn up until the relative late sampling in December, 1944, five months after initiation of the epidemic.

Since it was taken only on bush fowl or so-called bush turkey (*Megapodius*), there is no likelihood of this species having been concerned in the epidemic, and small possibility of its even being concerned in the disease cycle in nature. None was taken on any of the trapped local rats. However, a close relative, *A. innisfailensis,* was taken originally on rats in Queensland. In a few other endemic areas other members of this group have not been entirely above circumstantial suspicion as possible vectors (Kohls et al, l.c., p. 378; Philip and Woodward, 1946, p. 508).

Ascoschöngastia bushlandi, n. sp.

This species belongs in the large group of mites with swollen, clavate sensillae and unserrated chelicerae widely distributed in the Pacific area, and formerly assigned to *Neoschöngastia* of authors (not Ewing). The generic position of this large group of mites is still not settled, so that the present species is only tentatively assigned to the recently proposed *Ascoschöngastia* Ewing 1946, pending revision by others.

TABLE 1.—*Observed range and mean of Standard Data (Womersley) of scutal and setal measurements in μ of 30 specimens in type series (hs = humeral setae; ads = anterior dorsal setae of row 2)*

	Scutum						
	AW	PW	SB	ASB	PSB	SD	AP
Max.	55.1	89.9	23.2	27.5	24.6	50.7	31
Min.	47.8	70.3	15.2	22.4	18.2	41.4	27
Mean	51.6	81.3	21.4	25.0	22.0	46.9	28.8
Holotype	53	83.2	21	24.6	23.2	49.8	29

	Setae						
	am	al	pl	sens	hs	ads	vs
Max.	35.2	68.6	60.9	34.8	48	44.8	32
Min.	26.1	46.0	52.2	29	41.6	37	22
Mean	30.1	60.3	55.7	31.4	43.9	42.0	...
Holotype	30.4	61	53	34.8	46.4	43.5	24.6–32

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Description of larvae: Shape ovoid, color of engorged larvae in alcohol, pale yellowish, probably pinkish in life. Size engorged to 486 × 320 μ. Striae plain, normal, not crenulated. Standard Data are given in Table 1.

Vestiture: Galeal setae, and the dorsal, lateral and ventral setae on palpal segment IV (tibia) nude; that on segment III (genual seta) with three to four robust branches; those on segments

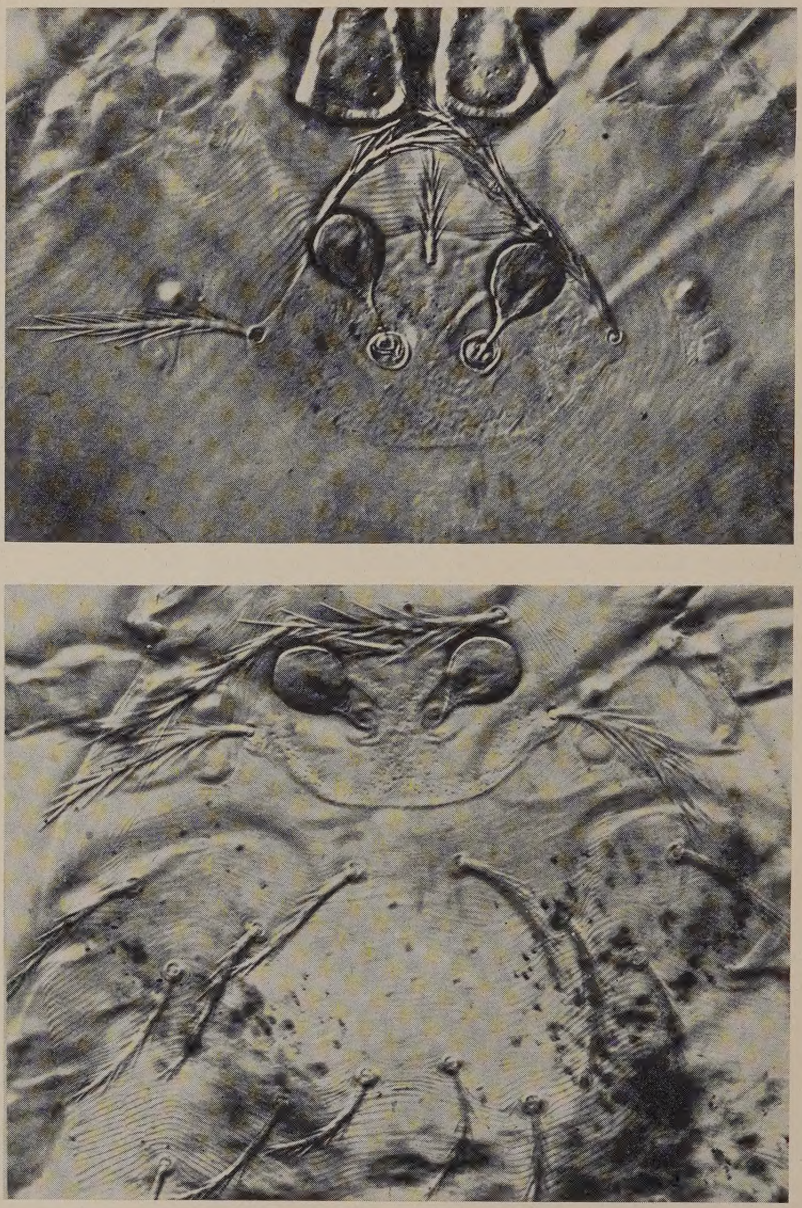

FIG. 1. The scutums of two paratypes of *Ascoschöngastia bushlandi*, n. sp. Sensillary bases partially everted in the upper specimen showing cup-like attachments at the bases of the stems. (Oblique-lighted microphotographs by N. J. Kramis, Hamilton, Montana.)

I and II (gnathosomal and femoral setae) plumose. Coxae unisetose; vestiture of remainder of legs not unusual; no long, nude seta on tarsus III. Scutal setae longer and more heavily ciliated than dorsal body setae (Fig. 1). The antero-median are the shortest, and antero-lateral usually the longest of the scutal setae (Table 1). The sensillae are rather deciduous and often loose in the mount, in which case a peculiar, small, cup-like structure at the base of the stem (Fig. 2) becomes evident, remindful of a miniature involucrum on the base of the stem of a "toad stool." This structure apparently provides the means of attachment and is seldom discernible in the deep, sensillary pores of sensillae *in situ*, except when occasionally the bases are everted. On detached sensillae they measure 2.9 to 4.1 μ in length. The terminal heads of the sensillae are capitate, subovoid, and minuately setulate, especially noticeable at the point where the stem expands into the head; in ten specimens, the means were 20.3 μ long by 15.5 μ wide. The dorsal setal counts

FIG. 2. Diagram of a detached sensilla and the vestiture of the capitulum of *A. bushlandi*.

are relatively constant, 2–6–6–6–6–4–2. A lateral pair may add to the fifth dorsal row in engorged specimens while the two lateral setae of the sixth row of 4 may become ventral in such well-fed mites. Total ventral setae, excluding the 2 pairs of sternal setae, approximate 31–36, with the shorter, preanal group numbering 18–23, and the longer postanals about 12–15. Setal measurements are given in Table 1.

Capitulum (Fig. 2): Chelicerae with the usual tricuspid caps and no teeth on the shafts. Cheliceral shields finely punctate; a transverse carina forms the hind margin connecting a sharp inner angle to an acute, robust, lateral tooth. Palpal claw trifurcate, though the third, short, basal prong is not visible in most preparations, which consequently appear bifurcate from the usual dorsal aspect. The main prong is unusually long and decurved.

Scutum (Fig. 1) with sensillae situated about on line with postero-lateral setae, and usually a little behind the midline of the scutum. Posterior margin broadly rounded and flattened behind. Anterior margin sinuous and broadly expanded in front of the antero-median setal attachment; lateral margins narrowly produced around the bases of each pair of setae. Sensillary pores large, separated by an interval subequal to their diameters and each recessed behind a short

diagonal, outwardly divergent carina. Finely punctate on the disc with evidence on many specimens of minute, irregular reticulations mesocaudad. Eyes 2 + 2, only the anterior with obvious corneal discs situated on ocular plates laterad of the postero-lateral angles of the scutum, and well separated from the scutum in engorged specimens.

Holotype and 29 *paratypes* (on separate slides) taken on bush fowl (*Megapodius*) in the rain-forest on Owi Island, adjacent to Biak, Schouten Group, Dutch New Guinea, 18 December 1944, by the author. Holotype and two paratypes in the U. S. National Museum No. 1780. Paratypes in collections of the British Museum (Natural History), South Australia Museum, the Rocky Mountain Laboratory, Dr. G. W. Wharton, and R. C. Bushland.

The holotype was selected with one attached, and one nearby, detached sensilla to show the basal structure on the stem which may or may not be unique for this species. It has not been described on any previous mites. In a revised key to the genus to be published by Womersley, *bushlandi* is closest to *innisfailensis* and *coorongensis,* but the cilia on the scutal setae are longer and scutal dimensions larger than in the former, and in both these species sensillary pores are described in front of line pl. In *coorongensis* al are shortest, pl are much the longest of the scutal setae, while in *bushlandi,* am are shortest and al are a little longer or subequal to pl.

It is a pleasure to name the species for Mr. (then Captain) R. C. Bushland, with whom the writer shared many vicissitudes during military service in New Guinea and the Philippine Islands, including mite-control work during the epidemic on Owi Island where this species was discovered.

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A NEW MITE, *EUBRACHYLAELAPS CROWEI*, FROM THE GRASSHOPPER MOUSE, *ONYCHOMYS LEUCOGASTER ARCTICEPS* (RHOADS)

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A small collection of mites from the grasshopper mouse *Onychomys leucogaster arcticeps* (Rhoads), represents an undescribed species of the family Laelaptidae. This new species is characterized by its almost circular shape, the reduced shoulders, and the reduced genito-ventral plate which bears a single pair of long setae. It is provisionally assigned to the genus *Eubrachylaelaps* Ewing.

Eubrachylaelaps crowei, n. sp.

Female

Outline of body almost circular, about 0.675 mm long and 0.550 mm wide. Shoulders obscure. Body sparsely clothed with setae on the dorsal side. Setae stouter ventrally. Legs short and stout.

Dorsal side: Dorsal plate almost covering the dorsum, with a slight projection anteriorly and rounded posteriorly. Peritreme sinuate, extending from near the posterior border of coxa III to the anterior border of coxa I.

Ventral side: Sternal plate rectangular, about one half as long as wide, and bearing three pairs of setae. Size and position of sternal setae and position of sternal pores as illustrated. Anterior border of sternal plate with a low hump, posterior border slightly concave. Genito-ventral plate with one pair of setae. Sides of genito-ventral plate almost parallel, its greatest width not exceeding the distance between coxae IV. Anal plate rounded anteriorly and with a dull point posteriorly. Paired anal setae about half the size of the unpaired seta, and placed closer to the posterior than to the anterior border of the anus. Metapodal plates small, oval.

Legs: Coxae I bearing two setae, the proximal one being heavier than the apical one. Coxae II with a basal seta on a slight projection of the anterio-medial border, and with a posterior apical seta. Coxae III with an anterior basal seta and a posterior seta, the latter being the shorter and heavier of the two. Coxae IV with a reduced mesal seta. Setae on other segments as illustrated.

Gnathasoma: Gnathasoma with four pairs of setae as illustrated. The chelicerae are pincer-like, the movable chela extending beyond the fixed chela and bearing three teeth in addition to the terminal process.

Measurements: Body, excluding mouthparts, 0.672 mm long and 0.553 mm wide; sternal plate, 0.102 mm long and 0.205 mm wide; anal plate, 0.113 mm long and 0.102 mm wide; dorsal shield, 0.668 mm long and 0.550 mm wide.

In the collection of the type series only females were obtained.

Types: Holotype female, U. S. National Museum No. 1779. Four paratype females are deposited in the U. S. National Museum, one in the collection of the Department of Entomology, Cornell University, and one in the collection of the author.

Collection data: The type series was collected from the grasshopper mouse, *Onychomys leucogaster arcticeps* (Rhoads), at the Rexroad Ranch, 13 miles southwest of Meade, Meade County, Kansas between July 9 and August 4, 1940. The collectors are John Eric Hill and Peter Emmet Crowe.

Comments.—Eubrachylaelaps crowei can be separated from *E. hollisteri* (Ewing) by a comparison of the sternal plates of the two species. That of the latter species is strongly arched posteriorly whereas *crowei* has the sternal plate only slightly concave posteriorly.

This mite bears the name of Peter Crowe, a promising mammalogist who was killed in action in the last war. For assistance in diagnosing this new species I am grateful to Dr. E. W. Baker and Dr. R. W. Strandtmann. For details regarding

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the collection of the type series I wish to thank Dr. J. E. Hill. Dr. C. Donald Grant has assisted the writer by sending a series of *E. hollisteri* from near the type locality.

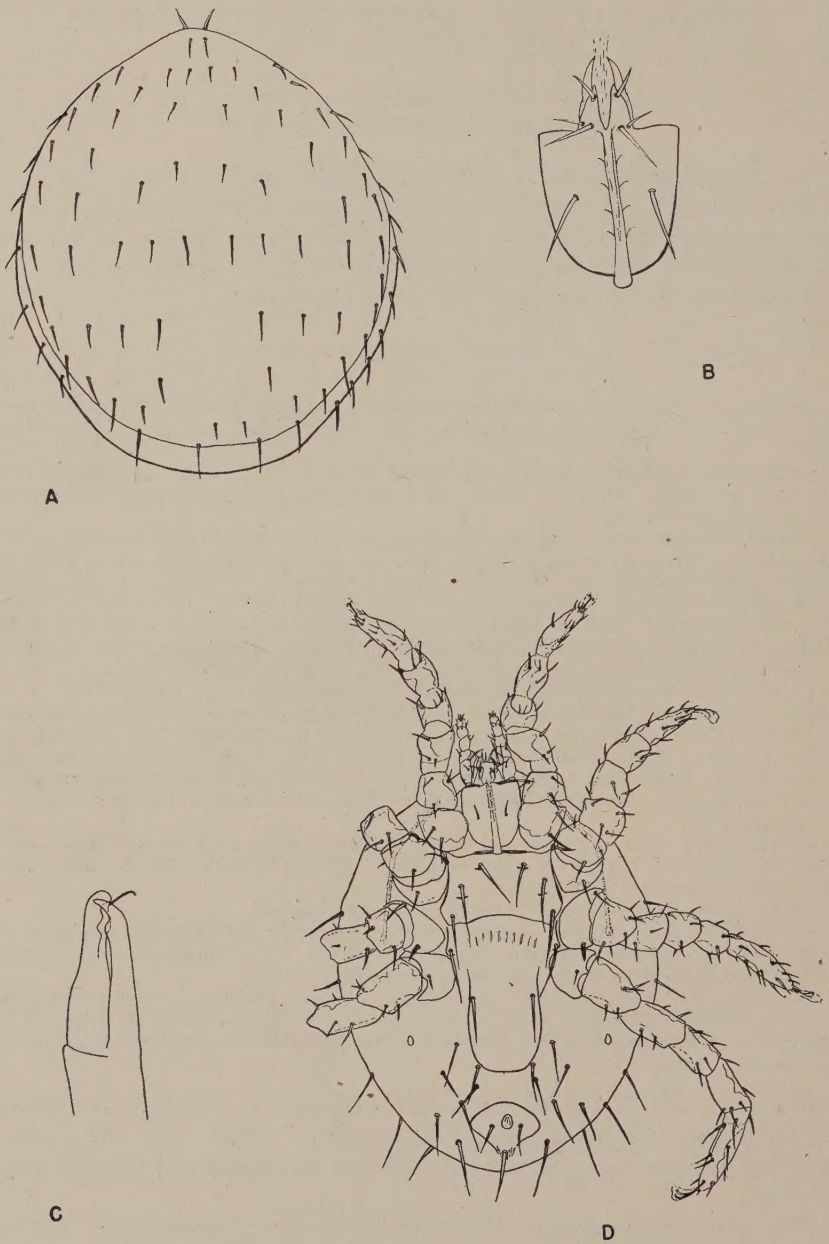

FIG. 1. Female of *Eubrachylaelaps crowei*, n. sp. A, dorsum; B, ventral view of gnathasoma; C, chelicera; D, venter.

LETHAL EFFECTS OF CECAL CONTENTS FROM CHICKENS INFECTED WITH CECAL COCCIDIOSIS AND THE INHIBITION OF THESE EFFECTS WITH IMMUNE SERA[1]

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INTRODUCTION

It has been previously reported (Wolfe, Bradford, and Herrick, 1946) that a saline extract of the homogenized lining of the chicken cecum, together with some connective tissue, when injected intravenously into chickens caused rapid death due to intravascular coagulation of the blood. Death occurred regardless of whether the tissue was taken from normal chickens or from those infected with the coccidia *Eimeria tenella*. It was also shown that antisera against the cecal, brain and lung tissues of chickens could be developed in rabbits and these antisera when incubated with the cecal tissue neutralize its *in vivo* clotting effects.

In the course of these previous experiments, the injection of cecal contents from some of the same chickens from which cecal tissue was taken was tried as a control measure. The contents were treated in exactly the same manner as the tissue itself. Normal chickens when intravenously injected with the cecal contents of other normal chickens regurgitated, defecated, and grew remarkably weak, but they recovered within ½ hour. If these test birds were killed for autopsy immediately after injection, no clots were found in any major vessels. On the other hand, when bloody cecal contents from chickens with cecal coccidiosis were treated similarly and injected into normal birds, rapid intravascular coagulation and death resembling that caused by the tissue extract ensued. This effect was attributed to a thromboplastin.

The main purposes of the present experiments were: (1) to determine when, in the course of the coccidial infection, the thromboplastic effect appeared in the cecal contents, and (2) to study the effects, on these contents, of antisera which were known to neutralize the clotting action of homogenized extracts of ceca, brain and lungs. An attempt was also made to determine whether the effects were more often fatal in infected than in the normal birds.

MATERIALS AND METHODS

To determine the time of appearance of the thromboplastic-like substance in the cecal contents, two separate groups of chickens, raised until the beginning of the experiment under coccidia-free conditions, were studied during the entire course of the infection. The weights of the chickens of one group ranged from 400 to 750 grams and the 2nd group from 250 to 400 grams. The procedures followed for each group were identical but in the first group the experiment was of shorter duration.

All of the chickens of each group were infected with approximately 90,000 sporulated oöcysts of *E. tenella*. On the day following the infection and on succes-

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sive days, up to 9 days in one group and 15 days in the 2nd group some infected birds were killed and the cecal contents removed. These contents were mixed with an equal volume of 1.8% saline and homogenized in a glass homogenizer. The material was centrifuged and the resulting supernatant fluid injected intravenously into one normal chicken and one chicken infected at the same time as the bird furnishing the cecal contents. In all cases, the condition of the mucosa and of the material itself was recorded as the cecal contents were removed from the infected birds. The usual dose of fluid was 1.0 cc although, in certain cases, a smaller dose was used. At all times the normal chicken chosen was of about the same size as the infected one selected for injection. All of the birds which died as a result of the injection were autopsied and examined for any clots in the major vessels.

In order to study the effects of antisera on the cecal contents, rabbits were injected intravenously with extracts of cecal mucosa, or lung or brain tissues. The procedures used in this antisera production were described in a previous paper (1946). These antisera resulted from at least two separate series of injections and the animals were bled 7 to 8 days after the last injection of the series.

Antisera so prepared were mixed in 1 : 1 proportions with saline extracts of the cecal contents. The mixture was incubated in a water bath at 37° C for 1 hour. In half of the cases, the mixtures were centrifuged and supernatant fluid injected immediately into normal chickens; in the others, the incubation mixture was kept over night in a refrigerator and then centrifuged and injected. The actual amount of cecal fluid injected was twice that determined as the minimum lethal dose (MLD). The MLD was determined separately for each collection of cecal contents used for incubation. As a control, normal rabbit sera were used in place of the antisera.

It was found that the bloody cecal contents rapidly lost their thromboplastic effects when frozen or stored for any length of time. Injections and incubations reported here were consequently made either on the day in which the cecal contents were harvested or on the day immediately following. In all cases, samples of the particular collection of cecal contents used for the incubations were tested for *in vivo* thromboplastic effect at the same time the incubated mixtures were injected to make sure of the potency.

RESULTS AND DISCUSSION

The Appearance of the Thromboplastic Effects in the Cecal Contents

The data presented in Table 1 show that the injection of fluid from cecal contents of chickens infected with *E. tenella* does not cause intravascular coagulation in the tested bird through the 4th day after infection. The fact that, in Group II, 3 infected test chickens and 1 normal test chicken died when injected with such cecal fluid may be attributed to the extraordinary sticky consistency of the fluid. No clots were found in these birds at autopsy carried out immediately after death. Symptoms were similar to those found in the previously reported experiment in which a saline extract of chicken pancreas was injected; death was slow and occurred without the muscular tetany typical in birds injected with thromboplastin.

All the chickens that received bloody fluid from cecal contents removed after the 4th and through the 13th day died except one. A normal chicken injected with 0.5 cc of the homogenate of the 11th day contents survived but an infected one in-

jected with 1.0 cc (not shown in table) died. Sufficient material was not on hand to repeat the test with a larger dose.

The hemorrhage which occurs on the 5th day of the disease results from a disorganization of the cecal mucosa brought about by rupturing schizonts. All chickens which died after injection with the cecal contents removed on the 5th day after infection through the 13th day were found to have intravascular clots. The presence of the thromboplastic effect, then, is concomitant with the severe disorganization of the mucosa on the 5th day and conceivably is due to the presence of tissue juices in the contents. The lysis of blood cells as well as of the cells of the cecal mucosa could possibly contribute to the amount of thromboplastic substance found in the blood of the cecal contents.

During the course of recovery from coccidiosis, a hard core, mainly of fibrin, may form in the cecum. The red color present at first because of the hemorrhage,

TABLE 1.—*Effect of intravenously injecting infected and non-infected chickens with hemorrhaged blood resulting from cecal coccidiosis*

Donors		Test animals					
		Group 1			Group 2		
Days after infection	Condition of cecal contents	cc inj.	Results of injection		cc inj.	Results of injection	
			Infected	Non-infected		Infected	Non-infected
1	Normal	1.0	None	None	1.0*	Lethal†	Lethal†
2	"	1.0	"	"	0.5	None	None
3	"	1.0	"	"	1.0*	Lethal†	None
4	"	1.0	"	"	0.5*	" †	None
5	Bloody	1.0	Lethal	Lethal	0.5	"	Lethal
6	"	1.0	"	"	0.5	"	"
7	"	1.0	"	"	0.5	"	"
8	Red core	1.0	"	"	0.5	"	"
9	" "	1.0	"	"	0.5	"	"
10	Red fading	..	.....	.....	0.5	"	"
11	" "	..	.....	.....	0.5	"	None
12	" "	..	.....	.....	0.5	"	Lethal
13	Almost white	..	.....	.....	0.5	"	"
14	White core	..	.....	.....	0.5	None	None
15	" "	..	.....	.....	0.5	"	"

* Material injected viscous with fine sediment that did not centrifuge out.
† No signs of intravascular clotting.

gradually disappears from this core; it is often entirely gone before the expulsion of the core which commonly occurs between the 10th and 15th day after the infection. At times when the core is very small it may be extruded earlier than the 10th day and such a core may be white. The mucosa gradually reorganizes and heals. Table 1 shows that in group II on the 14th and 15th days after infection, the cores were entirely white, and no coagulation occurred in the chickens injected with the homogenized core material. The mucosa was healing at this time, and it is logical to assume that the absence of the thromboplastic effect was due to the destruction or elimination of certain breakdown products from the site of injury.

The fact that during the whole course of the experiments more of the infected birds died after injection when treated exactly the same as their normal partner for the same day, probably indicates a weakened physical condition in the infected ones. There is no evidence from the *in vivo* experiments to indicate that the blood of infected chickens is any more or less coagulable than that of normal birds, although there is suggested evidence from several preliminary *in vitro* clotting experiments, that blood of chickens infected with *E. tenella* is more readily coagulable after the hemorrhage on the 5th day than the blood of normal chickens. This would agree

with results given by other authors for other animals. Shafiroff et al (1943), Drinker and Drinker (1915), Gray and Lunt (1914), von den Velden (1909) and many others have noted an increased coagulability of the blood after severe hemorrhage. Gray and Lunt stated that, in cats, the blood loss must be moderately severe to increase coagulability noticeably. They set 13% of the body blood as an amount of loss which gave an appreciable change in clotting time. Von den Velden found hypercoagulability resulting from loss of 40 cc of the circulating blood in rabbits and after loss of 400–500 cc in man. In chickens infected with coccidiosis, the blood loss is much more than 13%; Pratt (1938) found a loss as high as 37%.

The presence of a powerful thromboplastin in the cecum after the rupture of the mucosa in coccidiosis is suggested, not as the primary cause of death due to coccidial infection, but it may be a possible contributing factor in such deaths if it is reabsorbed into the blood. When as little as 1/10th cc of the fresh core extract, invariably kills normal, healthy chickens weighing over 800 grams, it is conceivable that a much smaller dose could be a factor in the death of the infected chicken which is in a generally debilitated condition.

On the contrary, there is also a possibility that small quantities of thromboplastic-like substance entering the blood over a period of time would keep the blood in a continuous negative clotting phase, since artificial introduction of a sublethal dose makes the blood incoagulable for a time. If hemorrhage itself affects the coagulability of the blood in dogs and humans to the extent that it is an important item in the pathogenesis of spontaneous venous thrombosis observed in infective and postoperative states as has been suggested, there is a reason to suppose that it might affect chicken blood similarly. Shafiroff et al (1943) have determined that the increase in coagulability is due to mobilization of thromboplastin in the blood. In such a case, perhaps the hemorrhage itself would induce production of emboli in many cases of coccidiosis without the necessity for adding a thromboplastin from the bloody cecal contents.

No work has been done on a possible mechanism for the introduction of the thromboplastin from the cecal contents back into the blood stream of the infected birds under normal conditions. Perhaps the breakdown of the epithelium would furnish enough of a circulatory entrance. Speculations only, not conclusions, are in order because of lack of experimental evidence and lack of knowledge of the substances discussed. The definite fact is that there is a thromboplastic effect produced by the cecal contents of chickens infected with *E. tenella* after the hemorrhage occurs. It is also a fact that this material is extremely powerful, amounts as small as 0.01 cc often killing perfectly normal chickens (800 grams) when intravenously injected.

The Effect of Antisera on the Cecal Contents of Infected Chickens

Table 2 records the data resulting from incubations of cecal contents of infected birds with certain antisera developed in rabbits. These antisera prepared against chicken cecum, lung, brain, and blood serum inhibited intravascular clotting activity of bloody cecal contents of infected chickens when the two were incubated and injected together. These results resemble closely those for the thromboplastic-like substance of the cecal mucosa itself, since antisera against chicken brain, cecum, and lung also nullified its clotting activity when similarly treated (Wolfe, Bradford and Herrick, 1946).

That the anti-chicken serum produced in rabbits did inhibit the *in vivo* activity of the core material and did not similarly inhibit that of the cecal mucosa extract has no definite explanation. A factor that may be important is that the so-called thromboplastic substance in the core material has a greater lability. Fluid from the cores loses its toxicity slightly after incubation at 37° C for 1 hour, and often the toxicity is markedly reduced after storage in the refrigerator for over 3 days. There was no reduction in potency of the cecal tissue extract after similar incubation, and storage for 3 days did not reduce measurably its thromboplastic effects. Presumably the greater lability of the thromboplastin from the cecal contents was due to the action of proteolytic enzymes present in the material upon fibrinogen or recalcified plasma for it was lysed when allowed to stand overnight. Clots formed by action of cecal mucosa extract upon the same materials did not lyse for 2–3 days. Clots formed by the action of chicken brain extract upon fibrinogen did not lyse when kept for 2 weeks.

A second factor that may be of importance is that the cecal contents of infected birds are very rich in blood serum and the thromboplastic factor may be adsorbed

TABLE 2.—*The neutralizing effect of anti-chicken tissue sera on bloody cecal contents*

Chicken tissue used for antiserum production	Number of chickens used	Effect of bloody cecal contents after incubation with antisera	
		Lethal	*Non-lethal*
Cecal mucosa	7	1	6
Lung	6	0	6
Brain	5	1	4
Cecal mucosa and lung	4	0	4
Blood serum	4	0	4
Control*	6	5	1†

* Normal rabbit serum.
† Extremely heavy chicken tested.

onto the serum proteins. An antiserum against the blood proteins could thus precipitate the lethal factor along with the precipitation of the serum protein.

There is little doubt that the thromboplastin obtained from the bloody cecal cores of infected chickens is similar in specificity to that extracted from the cecal mucosa of either infected or normal chickens. That there is a certain degree of organ specificity of thromboplastins as well as the species specificity has been shown by many authors and we have similar evidence (Wolfe, Bradford, and Herrick, unpublished data).

SUMMARY

1. When the cecal contents of chickens infected with *Eimeria tenella* were removed during the first four days of infection and subsequently intravenously injected into normal and infected chickens, they did not cause intravascular coagulation.

2. Intravenous injection of similar fluid after the 4th day of infection (after the hemorrhage occurs) through the period during which red color remains in the core material caused intravascular coagulation and rapid death in both the infected and normal chickens.

3. The intravascular coagulation was attributed to the injection of thromboplastin similar to that obtained from extracts of chicken cecal mucosa. The appearance of the thromboplastin in the cecal contents is correlated with the severe disorganization of the mucosa on the 5th day of infection.

4. The thromboplastin present in the cecal contents of infected birds after

hemorrhage on the 5th day of infection was similar to that from saline extracts of cecal mucosa as shown by the fact that both were neutralized by rabbit antisera prepared against chicken cecal mucosa, lung, and brain. The thromboplastic effects of the cecal fluid were also neutralized by a rabbit antiserum against chicken serum, while that from the cecal mucosa itself was not appreciably weakened.

5. The thromboplastin of the cecal material was more labile than thromboplastin prepared from the cecal mucosa, presumably because of proteolytic enzymes present in the material.

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SEX OF HOST AS A FACTOR IN CHAGAS' DISEASE

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Symptoms of Chagas' disease appeared more severe in males than in females during the course of routine subinoculation of *Trypanosoma cruzi* carried in mice. Since the literature contains no previous record of a sex-difference in susceptibility to *T. cruzi,* a quantitative check was undertaken.

MATERIAL AND METHODS

The data are based on a total of 152 infected mice of inbred stock designated as A-, C-, C3H-, and dba-strains. Males and females compared in this study were of uniform genetic constitution, age, and weight.

Results for three strains of *T. cruzi* are recorded. The "Reichenow" strain (R-strain), originally isolated from a patient in Brazil, was carried exclusively in mice by Dr. E. Reichenow in Hamburg from 1926–1936, by Dr. C. A. Hoare at the Wellcome Laboratories in London since 1936, and in our laboratory since February 1947. This strain is extremely virulent, and usually kills mice of all age groups within three weeks.

A culture of the considerably less virulent "Brazil" strain (B-strain) was obtained from Miss E. M. Johnson at the National Institute of Health, Bethesda, Md. The B-strain, derived from a patient in Brazil, was maintained at the National Institute of Health both in vitro and in rats for about six years. Since December 1945, we have kept one sub-line of this strain in culture and another in mice.

The past history of the "Culbertson" strain (C-strain), which is relatively avirulent for mice, is incomplete. In August 1936 Dr. J. T. Culbertson brought the material from the London School of Hygiene and Tropical Medicine to New York in an infected bug, *Rhodnius prolixus,* and propagated the trypanosomes for several years in albino rats (Culbertson and Kessler, 1942). A culture of the C-strain was made available to us in December 1946 by the National Institute of Health.

Infectious dosage was uniform in each experimental series. Infected blood (R-strain) withdrawn from the heart of a mouse at the height of infection was diluted with 5 volumes of sterile physiological saline, and test animals were inoculated subcutaneously with 0.1 cc of this preparation, i.e., about one-half million trypanosomes. *T. cruzi* from culture (B- and C-strains) was injected intraperitoneally in 0.5 cc amounts containing from 30–40 million crithidial forms of the parasite. The precaution of measured inocula was held necessary in this study, although Mazzotti (1940), working with a relatively wide range of dosage (800 to 8000 *T. cruzi*), found no corresponding variation in the severity of Chagas' disease in mice.

The data for blood-populations of *T. cruzi* are based on thick smears of peripheral blood taken from the tail-tips of infected mice at regular intervals. These preparations were made as uniform as possible. While counts made on thick smears cannot be regarded as absolute numerical values, errors resulting from variations in thickness would tend to be distributed evenly among males and females.

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The blood smears were air dried for 6–12 hours, and were stained and laked simultaneously for ten minutes in a solution of one part Giemsa stock (Hartman-Ledden Co.) in 20 parts of distilled water previously buffered by the addition of one Coleman buffer tablet (pH 7.00) to every 100 cc of water. Staining was followed by a quick rinse in un-buffered distilled water, and the slides were allowed to dry at room temperature. This procedure gave uniform results and preserved the otherwise rather fragile parasites intact. The individual counts represent surveys of 100 fields of thick blood smear with an oil-immersion objective at a magnification of 900 diameters.

Tissues and organs of infected mice were fixed in Zenker's fluid, paraffin-sectioned six microns thick, and stained with Mayer's hemalum and eosin.

RESULTS

The rise in blood-population of R-strain *T. cruzi* in 40 A-mice is plotted in Fig. 1. The hosts were eight weeks old at the beginning of this experiment. Care was taken

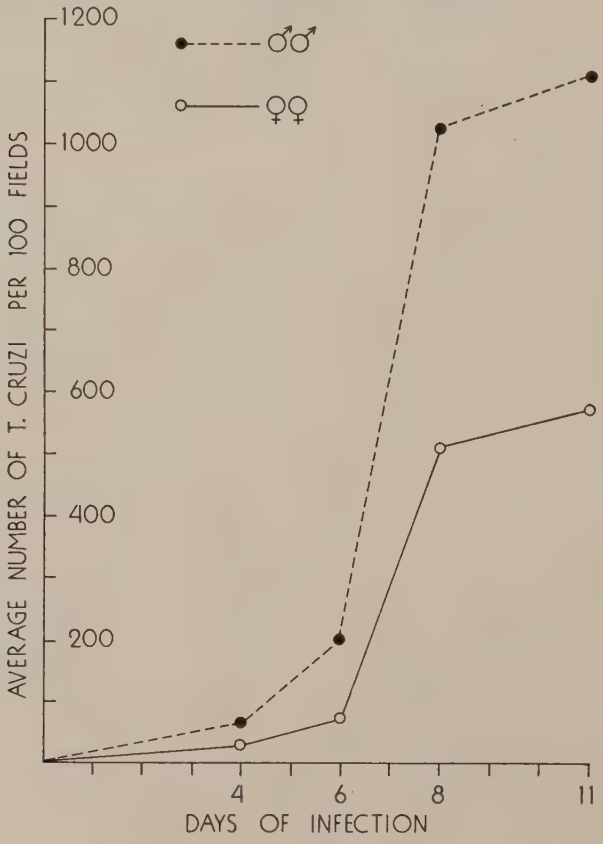

FIG. 1. Differences in blood population of *T. cruzi* (R-strain) in male and female A-mice, balanced as to litter, age, and infectious inoculum. Each curve is based on an initial lot of 20 mice. At 8 days, 18 males and 19 females survived. The two averages for the eleventh day are based on 8 male and 12 female survivors.

to compose the two groups of brothers and sisters. The curves are not carried beyond the 11th day, since at this time only eight males and twelve females survived. The pre-patent period was of approximately equal duration in the two sexes. The logarithmic phases of the two growth-curves coincide in time, but not in slope—the infection in the males attaining a level about twice that recorded for their sisters.

The consistency of this difference is shown in Table 1. Column II of this table gives, in order of size, the individual trypanosome counts corresponding to the eighth day averages plotted in Fig. 1. A similar consistent relationship held true for a group of 38 ten-week-old mice representing three kinds of inbred stock, as evident from Column I of Table 1.

A group of 10 male and 10 female C-mice (8–12 months old) averaged 1,568 *T. cruzi* for the males and 912 for the females per 100 microscopic fields at 900 diameters.

TABLE 1.—*Consistency of difference between* T. cruzi *blood populations in male and female mice**

I. R-strain *T. cruzi* in C3H-, dba- and white mice 7th day		II. R-strain *T. cruzi* in A-mice 8th day		III. C-strain *T. cruzi* in A-mice 3 weeks	
♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
11 780	2 550	7 020	3 960	23	16
10 260	2 320	1 700	1 950	13	8
8 680	2 260	1 530	550	11	5
4 160	2 200	1 300	460	5	4
3 440	1 770	1 080	400	4	2
3 180	1 740	1 050	400	3	2
2 430	1 650	1 020	360	3	2
2 210	1 560	960	350	3	2
1 980	1 510	650	240	2	1
1 820	1 460	650	210	2	1
1 760	1 240	540	210	1	1
1 510	1 150	530	200	1	1
1 490	1 050	440	100	1	1
1 440	970	330	90		
1 240	950	220	70		
1 230	850	200	50		
1 120	600	135	40		
1 070	470	60	35		
950	310		4		
Av. 3 250	Av. 1 400	Av. 1 022	Av. 509	Av. 5.5	Av. 3.5

* Figures are arranged in order of size and represent number of *T. cruzi* in thick blood smears per 100 high power fields.

Comparative counts obtained for the avirulent C-strain of *T. cruzi* are tabulated in Column III of Table 1. The number of mice in this series is too small for statistical significance, but the trend is in the same direction as in the preceding larger series.

Survival of 20 male and 20 female A-mice infected with R-strain is graphically represented in Fig. 2. All the males died within 14 days. At 15 days five females were still alive, two females survived to 20 days, one to 35 days. The B-strain of *T. cruzi* is less virulent than the R-strain. In a series of nine male and nine female A-mice (10 weeks old) infected with this strain, all males died within 43 days; three of the females survived indefinitely.

The rise of infection was usually accompanied by weight loss. Between the third and tenth day after inoculation 20 female A-mice lost an average of only 6.8 per cent of their initial body weight. Their male litter-mates lost 16.9 per cent during the same period. The larger blood-populations of *T. cruzi* in the male mouse could not be accounted for by parasitemia of the gonads. Sections through the testes of 6

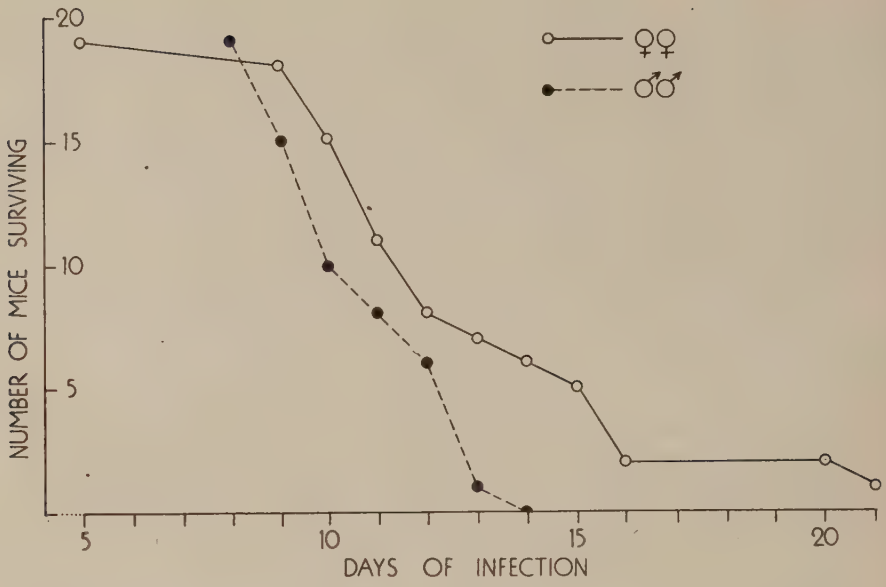

FIG. 2. Survival of male and female A-mice infected with *T. cruzi* (R-strain). Graph is based on 40 mice.

males infected with R-strain revealed only a few leishmanioid stages of *T. cruzi* in the encapsulating connective tissue. Histological study of sections through 6 ovaries likewise showed only scarce parasitism in the connective tissue. The germinal epithelium was never found parasitized in either sex. Table 2 lists the results of tissue examination of 4 male and 4 female mice of comparable age and genetic background infected with R-strain *T. cruzi*. The information in this table was obtained before I became aware of a sex difference, and hence bears the mark of complete objectivity. There is good agreement between the histological picture and the preceding result.

Statistical analyses of the counts from which Fig. 1 and part of Table 1 were compiled gave the following standard errors: the number of A-mice surviving on the sixth day of infection with R-strain was 20 males and 19 females. Only 27 per

TABLE 2.—*Tissue infection* (T. cruzi, *R-strain*) *in 4 male and 4 female mice of comparable age and genetic background*

Organ or tissue	Male mice				Female mice			
	–	+	++	+++	–	+	++	+++
Heart	2	2	–	–	4	–	–	–
Brain	–	–	–	–	–	–	–	–
Lung	1	–	3	–	3	1	–	–
Liver	1	–	–	3	–	1	2	1
Spleen	1	–	1	2	1	2	–	1
Kidney	–	2	2	–	1	3	–	–
Intestine	–	3	1	–	1	2	1	–
Skeletal muscle	–	–	1	3	–	2	1	1
Total	5	7	8	8	10	11	4	3

– = no parasites found in 100 high power fields.
+ = parasites rare.
++ = parasites present in about ¼ of the fields examined.
+++ = parasites abundant.

cent ± 7.1 per cent of the total trypanosomes counted occurred in female hosts. At 8 days, with 18 males and 19 females surviving, 33 per cent ± 7.7 per cent represents the female quota of the total trypanosome count. For the eleventh day (8 male and 12 female survivors) the corresponding figure is 34 per cent ± 10.3 per cent. In spite of the relatively small numbers of mice used in this study, the results are statistically significant. The consistency between parasite population in the blood and tissues, weight loss, and survival data is such that the sex difference in susceptibility to *Trypanosoma cruzi* may be considered a constant phenomenon.

DISCUSSION

While critical attention has been paid to species, genetic strain, age, weight, and diet of experimental hosts, sex as an environmental factor in host-parasite relationship has been treated with comparative neglect throughout the literature in protozoan parasitology.

It needs no special emphasis that the profound physiological differences between the two sexes might well affect the course of any infectious disease. Differences in the carbohydrate-, lipoid-, and protein-metabolism of male and female mice have been observed by several authors. Deane (1942), for example, noted a considerable sex difference in the fat deposition in the livers of mice placed on a high sugar diet. When a balanced diet was restored, the female livers returned to a normal histology more slowly than male livers. The rate of disappearance of subcutaneous vegetable oil was more rapid in male than in female mice, but this difference was abolished by castration before puberty (Turner and Mulliken, 1941). Wicks (1941) found that healthy male mice excreted large amounts of a peculiar urinary protein, whereas the females exhibited little or none. Metabolic differences such as these could exert stimulating or retarding influences on the life-cycle of *T. cruzi* which multiplies in a number of host tissues, including the liver.

A parallel can be drawn between the *T. cruzi* observations and the behavior of certain transplantable tumors. The latter may be looked upon as parasitic in the sense that they represent growth of foreign cells at the expense of a host. Gross (1941) studied the influence of sex on the evolution of transplantable sarcoma 37 in experimental mice. The incidence of "takes" was significantly higher in males than in females. There also was a striking difference in the incidence of tumor regressions which was 49 per cent in 182 females, and only 15 per cent in 207 males.

Generalizations concerning the "weaker sex" with regard to disease should be refrained from, since the female may be more susceptible than the male in certain instances. Ascoli (1946) found female albino rats much more susceptible to *Brucella abortus* than males, the mean lethal dose for females being about 1/2 to 1/3 that required for males. In this case, the particular tissues attacked by the infection were probably a decisive factor.

Recently, Burtt (1946) carried out transmission experiments with the tsetse fly, *Glossina morsitans,* and *Trypanosoma rhodesiense.* He found about twice as many male as female flies developing salivary gland infections. Although the male and female flies emerged in equal proportions from the pupae, the females did not thrive well under the conditions of mass cultivation in Bruce fly boxes. A greater proportion of potentially susceptible female flies may have been weeded out by early death, and this could account for a sex difference in infection rates. Burtt is now

attempting to settle the question of the relative susceptibility of the two sexes by experiments with flies kept singly in culture bottles.

Future experiments designed to elucidate the influence of hormonal and metabolic factors on the life-cycles of various trypanosomes in mammalian as well as invertebrate hosts are indicated and may prove fruitful.

Aside from many interesting theoretical implications, the sex difference in susceptibility to *T. cruzi* can be practically applied by using male mice in preference to females for the preparation of antigens, increased yield of "endotoxin" (Hauschka, Saxe, and Blair, 1947), routine sub-inoculation of laboratory strains of *T. cruzi,* and class-room demonstration of infected blood. Sex of host should also be of importance in critically controlled drug tests.

SUMMARY

Male mice of the A-, C-, C3H-, and dba-stocks were more susceptible to three strains of *T. cruzi* than female mice of corresponding age and stock. This sex-influenced difference was consistent with regard to trypanosome populations in the blood, degree of tissue infection, loss of body-weight, and survival of hosts.

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THE IN VITRO EFFECTS OF PENICILLIN ON THE GROWTH OF *TRICHOMONAS FOETUS*

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INTRODUCTION

A great deal of work has been performed concerning the effects of antibiotics on bacteria, both in vitro and in vivo. In sharp contrast, very little investigation has been made of the possible effects of the various antibiotics on protozoa. The purpose of this research is to present data concerning the in vitro effects of penicillin on the population growth of *Trichomonas foetus,* a protozoan.[1]

T. foetus, a parasitic flagellate, was chosen as a representative protozoan because of the relative ease with which it can be grown on artificial media as compared to most pathogenic protozoa, and because it was easily obtainable in pure culture. It is the cause of bovine trichomoniasis (trichomonas disease, trichomonad abortion, trich or early abortion) and is found in the reproductive organs of cattle suffering from the disease. *T. foetus* is characterized by three anterior flagella, an undulating membrane, and an axostyle which runs through the entire body and projects as a short "tail." It is about 15 μ long by 6 μ wide.

Antibiotics have been used by some workers as a means of obtaining bacteria-free cultures of protozoa. Mahmoud (1944) used 30 units of penicillin per tube of medium and obtained bacteria-free cultures of *T. foetus.* Williams and Plastridge (1946) tried various antibiotics; among them were penicillin, streptomycin, clavacin, gramicidin and actinomycin. They found that clavacin, gramicidin and actinomycin, when added to the basic medium in concentrations sufficient to control bacteria, were toxic for *T. foetus.* They obtained better results with penicillin and streptomycin used in combination. Morgan and Campbell (1946), in testing 350 compounds for possible trichomonacidal effects, found that penicillin in a concentration of 8.0% (80,000 units per milliliter) killed *T. foetus* in one minute, and that tyrothricin in a concentration of 0.03% also killed *T. foetus* in one minute. Morgan (1946), in addition, used antibiotics in obtaining bacteria-free cultures of *T. foetus.*

The present investigation is designed to ascertain whether or not penicillin, in concentrations lower than the massive doses used by Morgan and Campbell, inhibits the growth of *T. foetus,* and if so, to demonstrate the critical range by means of population growth curves. Comparisons will be made between different manufacturer's products to determine any differential effect that may arise.

MATERIALS AND METHODS

Culture medium.—Two media were employed, one for maintaining the stock cultures and the other for use in the experimental work.

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[1] The penicillin used in this work was very kindly supplied by Dr. Henry Welch, Chief, Division of Penicillin Control and Immunology, Food and Drug Administration, Washington, D. C.

The stock culture medium was a modification of Schneider's (1942) citrate medium. It consisted of two phases, a slant and a supernatant. First a "citrate" solution was made consisting of: sodium chloride, 5.0 g; magnesium sulphate, 0.2 g; ammonium acid phosphate, 1.0 g; potassium phosphate (dibasic), 1.0 g; sodium citrate, 2.0 g; glucose, 10.0 g; and distilled water up to 1000 ml. The slant portion was prepared by mixing six whole eggs with 60.0 ml defibrinated bovine blood and 50.0 ml of the "citrate" solution. This mixture was shaken thoroughly with glass beads in a 2-liter flask. The suspension was then filtered through cheese cloth and distributed in 4–5 ml quantities into 18 × 150 mm Pyrex tubes and plugged with cotton. The tubes were slanted and inspissated in an autoclave at 15 pounds for 20 minutes.

The liquid supernatant was prepared, and consisted of: "citrate" solution, 1000 ml; bovine serum, 50–75 ml; 1.6% alcoholic solution of bromcresolpurple, 2.0 ml; hematin, 40.0 mg. After adjusting the pH to 7.2–7.4 with NaOH, the mixture was placed in a 2-liter flask, plugged with cotton and autoclaved at 15 pounds for 20 minutes. After cooling, portions, sufficient to cover the slants completely, were transferred aseptically to the tubes containing the egg slants.

The experimental work was performed in a fluid medium suggested by Plastridge (1943). It was prepared as follows: beef infusion, 1000 ml; peptone, 10.0 g; glucose, 10.0 g; sodium chloride, 5.0 g; agar, 0.7 g; and bovine serum (heat inactivated), 20 ml. This was adjusted with NaOH to pH 7.4, dispensed in 8.0 ml portions in 18 × 150 mm Pyrex tubes, plugged with cotton and autoclaved at 15 pounds for 20 minutes. After autoclaving, the final pH was found to be 7.1–7.2.

Organisms employed.—The trichomonad used in this study was *T. foetus,* strain "BR," originally isolated by Morgan and Wisnicky (1942) from a cow suffering from a trichomonad pyometra. It has since been kept in pure culture on the modified Schneider's medium.

Dilution and distribution of the drug.—The penicillin was obtained in 25-ml vials, each containing 100,000 or 200,000 International units in the form of a dried salt. The diluent used was the fluid medium in which the experimental work was performed. All dilutions were made in the original 25-ml vial and 2.0-ml portions were transferred to the tubes containing 8.0-ml portions of the medium, the combination resulting in 10.0 ml of medium containing the desired concentration of the drug.

Inactivation by "Clarase."—2.0 ml of a 5% solution of "Clarase" was added to the vial of penicillin for every 100,000 units present.[2] This was left standing at room temperature for 24 hours. After this time, the mixture was tested for activity on an agar plate seeded with *Staphylococcus aureus* (209P).

This inactivated residue was diluted as if the penicillin was present so that the resulting dilutions contained the same amount of residue present in the comparative dilution of penicillin.

Inactivation by penicillinase.—1.0 mg of Difco penicillinase per 3000 units was added in the form of a 1.0% aqueous solution and left standing at room temperature for 24 hours. The mixture was tested for activity on an agar plate seeded with *S. aureus* (209P). The inactivated residue was diluted as for the "Clarase"-inactivated residue.

[2] "Clarase" prepared by Takamine Laboratory, Inc., Clifton, N. J.

Inactivation by hydroxide.—2.0 ml of 1.0 N sodium hydroxide was added to the vial of penicillin for every 100,000 units present. This was left standing at room temperature for 24 hours. The mixture was tested for activity on an agar plate seeded with *S. aureus* (209P). The inactivated residue was diluted as for the "Clarase"-inactivated residue, after first neutralizing the mixture with 1.2 N hydrochloric acid.

Conditions of growth.—An inoculum of approximately 150,000 active trichomonads per ml was standard during the study. Every procedure, with the exception of the counting, was performed aseptically, and contamination was tested for daily by inoculating a loopful of the culture into nutrient broth. At the end of every series, films were stained and examined.

All cultures were incubated at 37° C, and uniform 18 × 150 mm Pyrex tubes were employed throughout.

Determination of population count.—The population counts were made with a Levy hemocytometer counting chamber. The culture tubes were rotated and 0.5-ml samples withdrawn and added to 0.5 ml of a 5% solution of formaldehyde. To this volume was added 4.0 ml of 0.85% saline, and after mixing thoroughly, a sample was withdrawn and pipetted under the cover glass of the hemocytometer. A count was made of all the organisms in each of the four 1.0 sq. mm sections and the average of these four readings was taken. This figure was multiplied by the total dilution factor, 100,000, and the count per ml was obtained. The average of the counts of both sides of the hemocytometer was taken as the final count.

All experiments were performed in duplicate and 14 samples were counted for each determination. The figures obtained agree to within 5.0% and exceed that number no more than once in twenty times.

At the time 0.5-ml samples were withdrawn from the culture tubes for counting, a separate portion was examined under the microscope. If approximately half the organisms were not motile, the culture was considered "non-motile" and counting was discontinued. However, where cultures are spoken of as being "sterile," no motile organisms were observed.

Determinations of pH were made with a Beckman pH meter.

RESULTS AND DISCUSSION

Four manufacturers' samples of amorphous sodium penicillin were tested against *T. foetus*. All four samples were inhibitory in their effect, with the critical range falling between 1000 u/ml and 2500 u/ml. Plate I shows the relative effects of the four samples on the population growth, and shows the variation of the critical range between the different samples. This inhibitory effect could be due to any one or combination of the following different fractions present in commercial amorphous sodium penicillin: 1) penicillin, 2) sodium ion with which the penicillin is associated, 3) penicillin breakdown products, 4) non-penicillin residue that is a result of the lysis of the mold mycelium and other factors inherent in the manufacturing process, or derivatives from the medium upon which the Penicillium is grown.

The amount of sodium ion present as NaCl per ml of the test medium is 19.5 mg, while the amount of sodium ion present per ml of a 2500 u/ml dose of penicillin is 0.09 mg. The addition of so small an amount of sodium ion to the relatively large amount, in the presence of which the organisms thrived, could hardly be detrimental.

To check this, however, the amount of NaCl in the medium was raised from 5.0 g to 5.1 g without any apparent effect on the organism. Therefore, the sodium ion was eliminated as a possible agent of inhibition.

Three manufacturers' samples of crystalline sodium penicillin, which is devoid of non-penicillin residue, were tested against *T. foetus*. Although they, too, exhibited an inhibitory effect, they differed from the amorphous penicillin samples in that the level of activity was appreciably lower, and that there was not as much variation between the different crystalline samples. This is shown in Plate II and Table 1. Whereas the critical range for amorphous penicillin was 1000 u/ml–2500 u/ml, it was 10,000 u/ml–20,000 u/ml for the crystalline. A concentration of 10,000 u/ml of crystalline penicillin barely approached the level of activity produced by 2500 u/ml of amorphous penicillin, while 10,000 u/ml of amorphous penicillin resulted in death within 24 hours.

TABLE 1.—*Showing the difference in activity levels between amorphous and crystalline penicillin and the relative variation between different manufacturer's samples of the two types of penicillin*

A. Amorphous sodium penicillin

Per cent of the maximal (48-hr.) population of the control		
Manufacturer	1000 u/ml	2500 u/ml
A	4.3%	Dead
B	72.3%	48.3%
C	65.5%	16.3%
D	58.7%	7.1%

B. Crystalline sodium penicillin

Per cent of the maximal (48-hr.) population of the control				
Manufacturer	1000 u/ml	2500 u/ml	10,000 u/ml	20,000 u/ml
A	73.0%	68.9%	43.1%	Not tested
E	77.8%	63.5%	45.6%	Not tested
G	83.6%	66.9%	50.9%	0.9%

From the above, three conclusions may be drawn. First, penicillin does inhibit the growth of *T. foetus*. Second, the difference in activity levels of the two types of penicillin is in some way connected with the non-penicillin fraction present in commercial amorphous penicillin. Third, the concentration of the causative agent of this apparent difference in potencies varies with the manufacturer and might possibly be due to something inherent in the process of preparing the product.

Three manufacturers' samples of amorphous sodium penicillin were inactivated by the addition of a 5% solution of "Clarase." All three samples displayed a marked inhibition for *T. foetus*, although the degree of inhibition varied with each manufacturer's sample. This is shown in Plate III. This inhibitory effect which was greater than that produced by the active amorphous sodium penicillin, could have been caused by one or both of the following: 1) penicillin breakdown products plus "Clarase," 2) the production of an unknown substance from the interaction of the "Clarase" and the non-penicillin constituents of commercial amorphous penicillin.

Four manufacturers' samples of crystalline sodium penicillin were inactivated by the addition of a 5% solution of "Clarase" and tested against *T. foetus*. In no case was any effect apparent. This ruled out the possibility of inhibition being caused by penicillin breakdown products plus "Clarase." There remained only the possibility of the action being caused by the unknown substance produced by the

interaction of "Clarase" and the non-penicillin fraction of commercial amorphous penicillin.

Two manufacturers' samples each of amorphous sodium penicillin and crystalline sodium penicillin were inactivated by the addition of Difco penicillinase and tested against *T. foetus*. No effect was apparent in either the amorphous or the crystalline penicillin.

Two manufacturers' samples each of amorphous sodium penicillin and crystalline sodium penicillin were inactivated by the addition of NaOH and tested against *T. foetus*. No effect was apparent in either the amorphous or the crystalline penicillin.

From the above data the following conclusions may be drawn: 1) the non-penicillin residue in amorphous penicillin has no effect upon the growth of *T. foetus*, 2) the inhibitory effect noted in the "Clarase"-inactivated amorphous penicillin was due to the interaction of the "Clarase" and something present in the non-penicillin residue of the amorphous penicillin products.

The difference in activity levels between crystalline and amorphous penicillin as shown, was in line with recent findings of the penicillin industry. A study of this was recently made by Welch, Randall, and Price (1947). They found that there was a "factor" present in amorphous penicillin which markedly enhanced penicillin

TABLE 2.—*The relative potency of crystalline penicillin held at 37° C in the test medium used in this study*

Time	Potency	% potency of original sol.
0 hrs.	2220.0 u/ml	100.0%
24 hrs.	1560.0 u/ml	70.3%
48 hrs.	945.0 u/ml	42.6%
72 hrs.	706.5 u/ml	31.8%

activity while being devoid of activity itself, that the "factor" enhanced the effectiveness of each of the fractions G, F, X, K and dihydro F, and that the "factor" was not inactivated by heating at 100° C for 48 hours nor by penicillinase.

Some of the population curves shown, particularly in the higher dosage levels, displayed a steeper slope in the second 24-hour period than in the first 24-hour period. This was attributed to the fact that penicillin loses a fraction of its potency when held at 37° C in the fluid medium used. Table 2 shows the relative potency of crystalline penicillin held at 37° C in the test medium.[3]

A variation in the peak population reached by the "controls" was observed to have a direct correlation with the medium batch used. It is suggested that this is due to variations in the quality of beef used in preparing the beef infusion, and to possible variations in the bovine blood that was obtained from local slaughter houses.

At various times during the course of this study, it was observed, particularly at the higher dosages after 48 hours, that the organisms swelled to almost twice their normal width. The average size of the swollen trichomonads was $15.7 \times 14.1\ \mu$, as compared with a normal of $15.9\ \mu \times 6.7\ \mu$. At first, it was thought that this swelling might be symptomatic of penicillin activity. However, one set of experiments failed after 36 hours because of a badly prepared batch of medium, and this swelling was noted here, too, just prior to death.

In addition to the swelling, many of these cultures were observed in which vacuole-like structures had appeared. Both the enlargement of the organisms

[3] This assay was made through the courtesy of Mr. Louis F. Ortenzio, Division of Penicillin Control and Immunology, Food and Drug Administration, Washington, D. C.

and the appearance of vacuole-like structures have also been observed by Morgan (Personal communication, 1947) and that worker is now engaged in morphological studies of this phenomenon.

Through good fortune, six different salts of amorphous penicillin were obtained. All these samples were prepared from the same master lot of penicillin so that the result was a fairly good picture of the relative cation effect. The salts obtained and tested were sodium, calcium, strontium, potassium, ammonium and magnesium. At the 2500 u/ml dose, the sodium salt exhibited relatively the same degree of inhibition as was observed in the previous tests of amorphous sodium penicillin, but all the other cations were toxic at that level, the cultures becoming sterile within 26 hours. At the 500 u/ml dose, an interesting set of curves was obtained, as shown in Plate IV. The calcium and potassium salts had approximately the same inhibitory effect as the sodium salt. The magnesium and strontium salts were appreciably more inhibitory than the aforementioned three. The ammonium salt displayed the greatest degree of inhibition. It seems interesting to note that the culture which was inhibited the most, grew most slowly and lived the longest. The control, sodium, calcium and potassium salts completed their cycle in 96 hours. The ammonium salt completed its cycle in 120 hours. The ammonium and strontium salts reached their peak population at 72 hours as compared with 48 hours for all the rest. As yet, the significance of this observation is not clear.

The pH changes with age were uniform throughout the course of the experiments. The initial pH was 7.2 and the terminal pH at the end of 72 hours varied from 4.73 to 5.21.

SUMMARY

1. It is here demonstrated that penicillin inhibits the population growth of *T. foetus,* in vitro.

2. The level of activity of amorphous penicillin as measured by the inhibition of population growth, is shown to be appreciably higher than the level for the corresponding dose of crystalline penicillin.

3. Evidence indicates that a "factor" is present in the non-penicillin residue of amorphous penicillin which greatly enhances the activity of the penicillin, although it has no activity by itself.

4. When amorphous penicillin was inactivated by the addition of "Clarase," the resulting combination was shown to be more inhibitory for *T. foetus* than any of the other substances tested.

5. In a test of the relative inhibition of six salts of penicillin, it was found that the ammonium salt had the greatest inhibitory effect on the population growth, although this culture lived one and two-thirds times as long as the control.

ACKNOWLEDGMENTS

For their generous and unfailing help, the author is profoundly grateful to Dr. Theodor von Brand, the National Institute of Health, Dr. William A. Randall, Mr. Clifford W. Price, Miss Velma L. Chandler and Miss Lila F. Knudsen, all of the United States Food and Drug Administration, Washington, D. C. Appreciation is also due to Dr. Banner Bill Morgan, University of Wisconsin, who kindly supplied the culture of *T. foetus* and helped with many suggestions, and to Dr. E. G. S. Baker, The Catholic University of America, for his many helpful suggestions and criticism.

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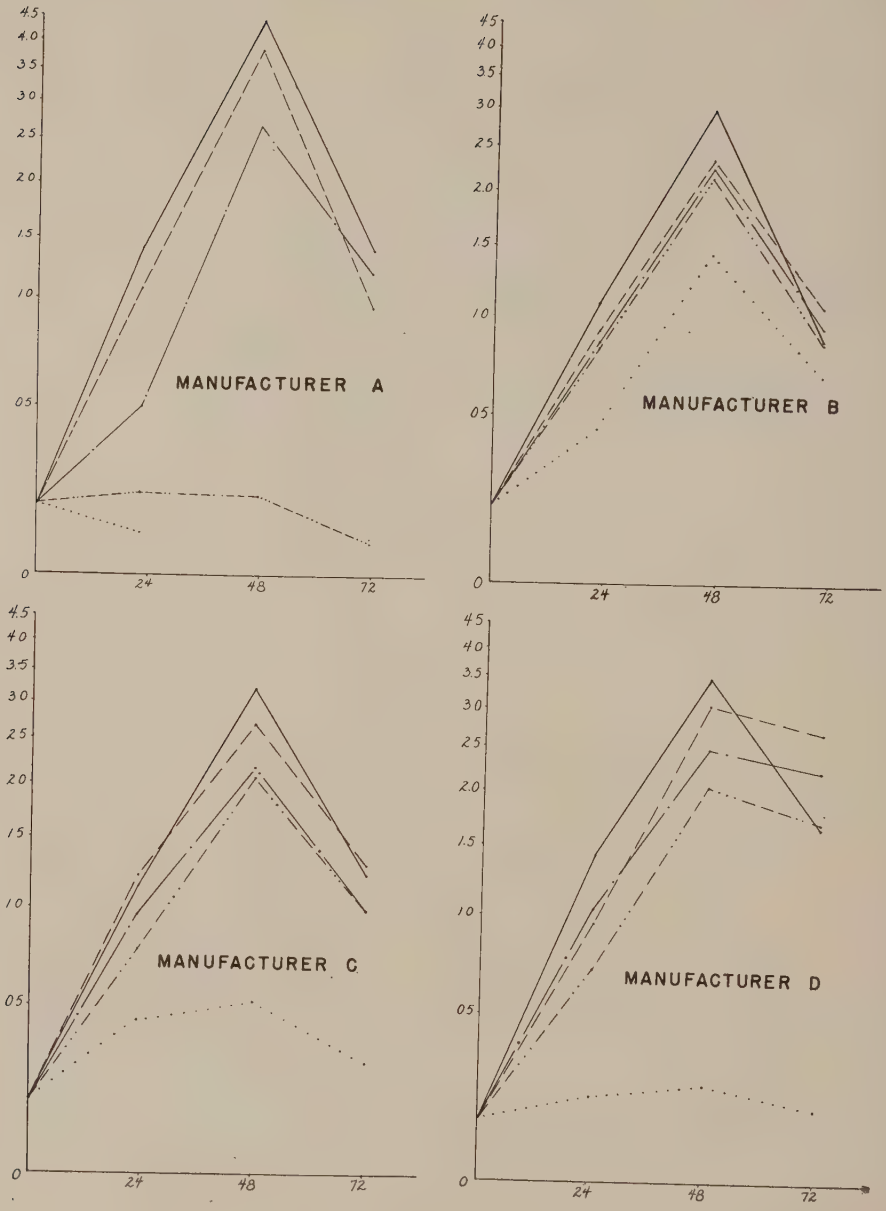

PLATE I

Population curves of *T. foetus* in various dilutions of four manufacturers' samples of amorphous sodium penicillin. The numbers along the abscissa represent the age of the culture in hours. The numbers along the ordinate represent the population count in millions of organisms per ml, plotted along a logarithmic scale.

Legend

——————— .....control
— — — — .....100 u/ml
•———— • .....500 u/ml
···— — — .....1000 u/ml
····· .....2500 u/ml

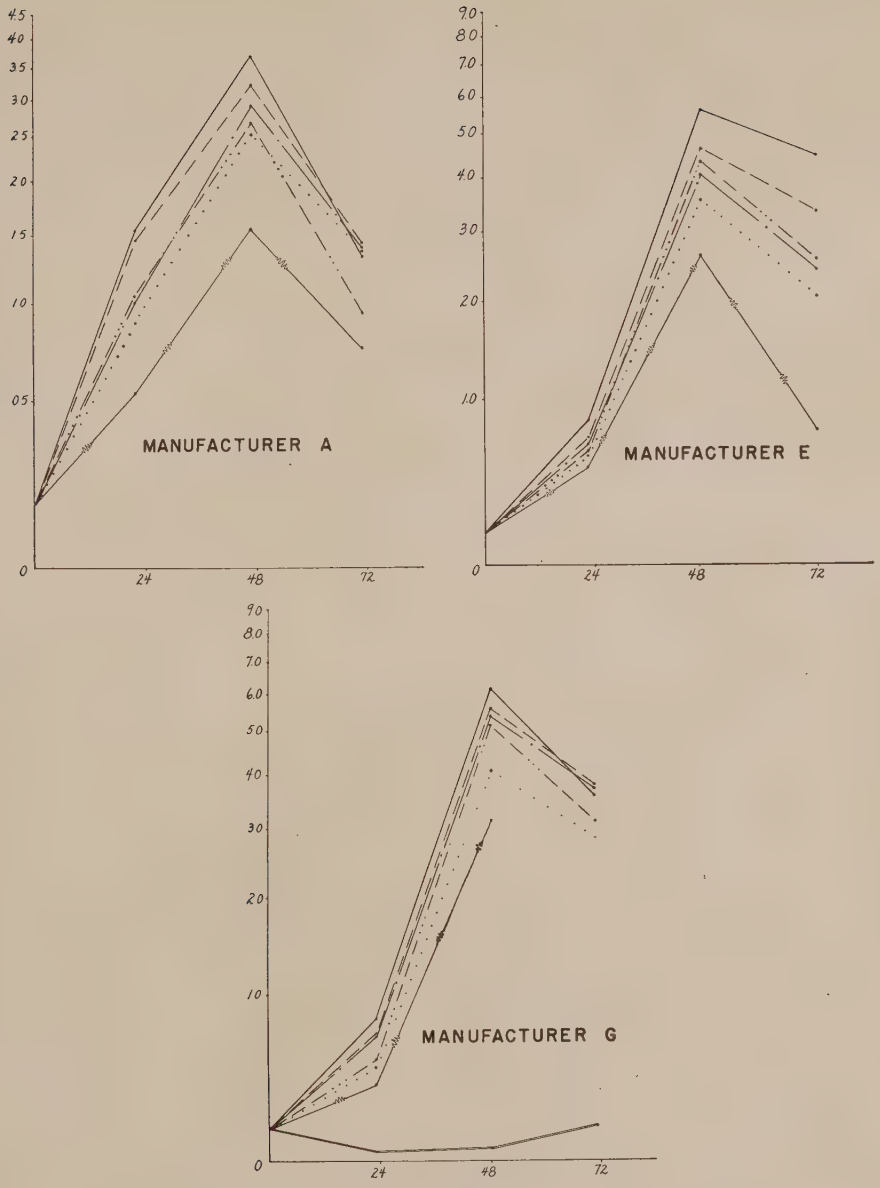

PLATE II

Population curves of *T. foetus* in various dilutions of three manufacturers' samples of crystalline sodium penicillin. The numbers along the abscissa represent the age of the culture in hours. The numbers along the ordinate represent the population count in millions of organisms per ml, plotted along a logarithmic scale. Note that the curves for manufacturer A are to a different scale.

Legend

- control
- 100 u/ml
- 500 u/ml
- 1000 u/ml
- 2500 u/ml
- 10,000 u/ml
- 20,000 u/ml

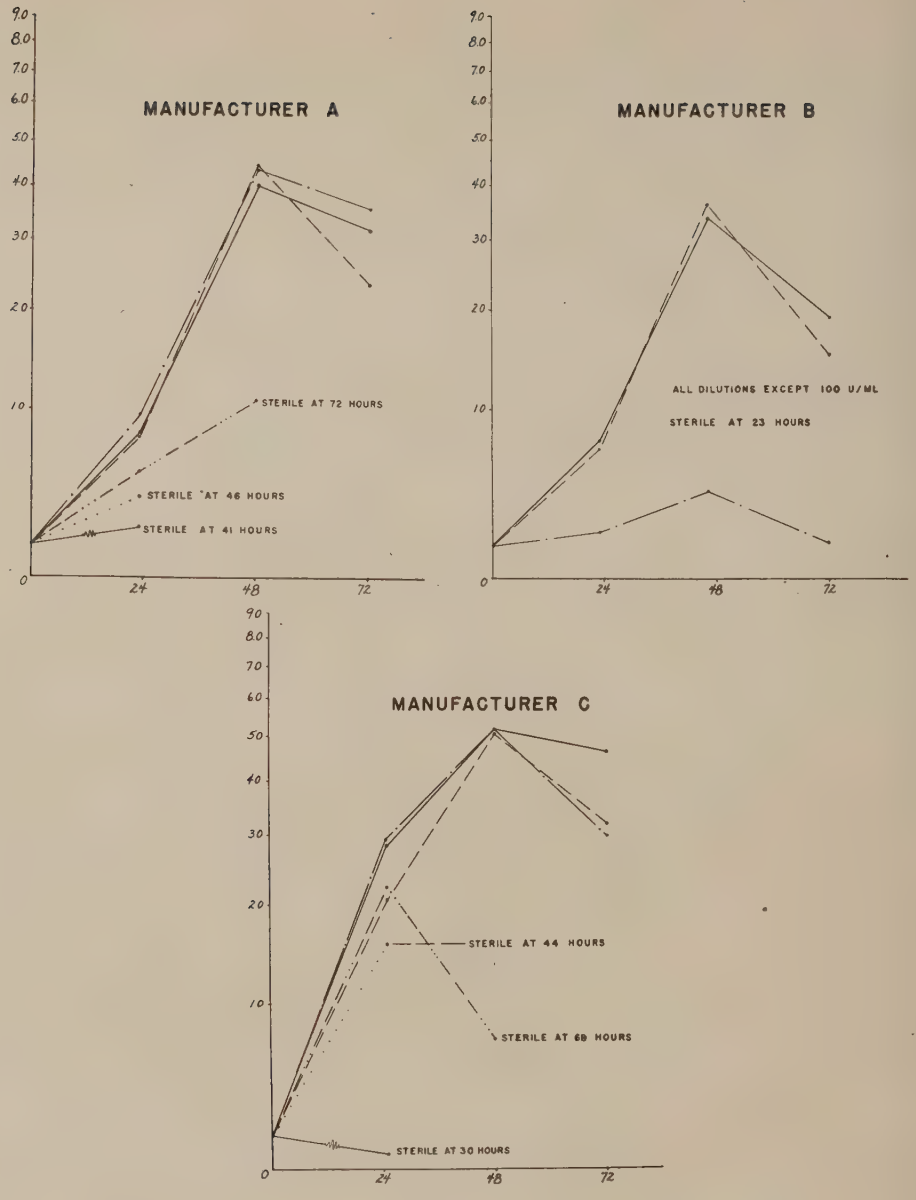

PLATE III

Population curves of *T. foetus* in various dilutions of three manufacturers' samples of amorphous sodium penicillin inactivated by the addition of "Clarase." The numbers along the abscissa represent the age of the culture in hours. The numbers along the ordinate represent the population count in millions of organisms per ml, plotted along a logarithmic scale.

Legend

control
"Clarase" control
100 u/ml
500 u/ml
1000 u/ml
2500 u/ml

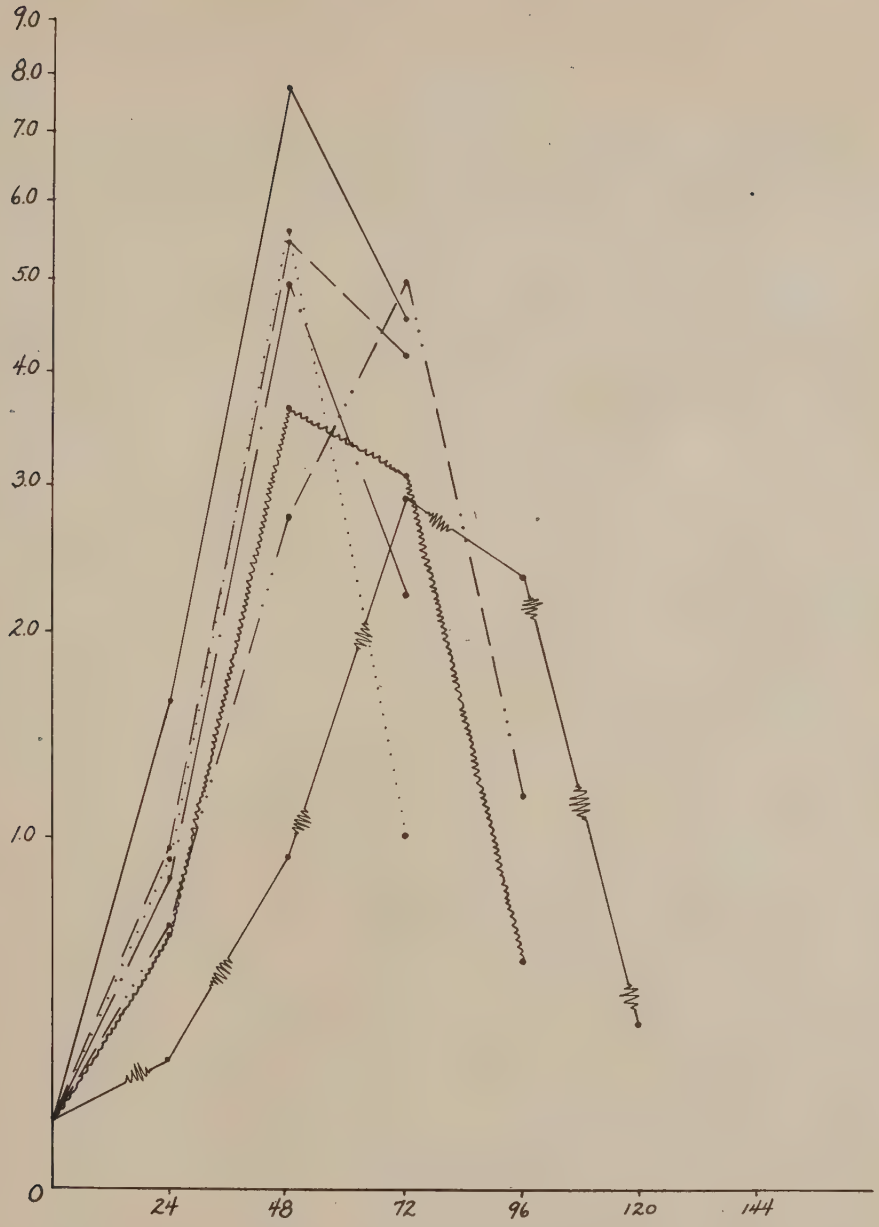

PLATE IV

Population curves of *T. foetus* in cultures containing 500 u/ml of six different salts of amorphous penicillin made from a single master lot. The numbers along the abscissa represent the age of the culture in hours. The numbers along the ordinate represent the population count in millions of organisms per ml, plotted along a logarithmic scale.

Legend

——————.....control
— — — —.....sodium
.———.....calcium
...— — —.....strontium
.....potassium
——/\/\—.....ammonium
~~~~~~~~~~.....magnesium
~~~~~~~~~~

THE PROLONGATION OF THE VIABILITY OF CULTURES OF *E. HISTOLYTICA* BY THE ADDITION OF STREPTOMYCIN

CLIFFORD L. SPINGARN, M.D., AND MORTON H. EDELMAN, M.D.[1]

Previous observations have indicated that streptomycin does not kill trophozoites of *Endamoeba histolytica* in vitro (Balamuth and Wubold, 1946). In studying the action of streptomycin on cultures of *E. histolytica,* we confirmed this finding and noted further that streptomycin actually prolonged the time of survival of cultures of trophozoites eliminating the necessity of frequent transplants to fresh media to maintain the strain. This report is a presentation of the results of our experiments.

METHODS

The strain of *Endamoeba histolytica* used in the experiments was obtained from the Tropical Disease Clinic, The Washington Heights Health Center, Department of Health of the City of New York. This culture of active trophozoites had been isolated on *Endamoeba* medium (Difco) from the feces of an individual infected with *E. histolytica.* In our laboratory, it was maintained by subculturing on the same medium every 48 hours.

The culture medium was prepared by dissolving 33 grams of *Endamoeba* medium (Difco) in 1000 cc of distilled water. The solution was tubed in 5 cc amounts and autoclaved at 15 lbs. pressure for 20 minutes. The tubes were allowed to solidify in a slanted position. Immediately prior to use, the slants were covered with 2 cc of a mixture of fresh sterile horse serum in normal saline (1:6). A small amount of rice powder (Difco) was then added to each tube by means of a 1-cc pipette.

After inoculation with 0.05 cc of the serum-saline containing trophozoites, the tubes were incubated at 37° C and were examined at 24- and 48-hour intervals for viable trophozoites. This was done by aspirating a small drop of serum-saline from the bottom of the tube and placing it on a clean slide under a cover slip. Amoebae, if present, were observed for size, motility, and the presence of phagocytized rice particles. Serum-saline (1:6) and rice powder were added to the cultures every 48–72 hours to replace losses due to removal, evaporation and phagocytosis.

The streptomycin used was dissolved in distilled water in a concentration of 10,000 units per cc and was added to the serum-saline, at the time of inoculation of a fresh culture tube, in amounts sufficient to give final concentrations of 1000, 2000, or 3000 units per cc. The potency of the drug was assayed at weekly intervals against a standard strain of bacteria.

EXPERIMENTAL

Forty-eight-hour-old cultures usually contained large numbers of motile amoebae (8–10 amoebae per high power field). These varied from 8 to 40 micra in diameter and usually contained 6–10 rice particles in their cytoplasm. Motility was indicated by the extrusion of pseudopodia and was maintained at room temperature for periods

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[1] From the Division of Bacteriology of the Laboratories of The Mount Sinai Hospital, New York.

of over 1 hour. All the culture tubes showed evidence of bacterial growth as indicated by the turbidity of the serum-saline overlay, the presence of colonies on the slant, a flaky pellicle at the surface of the serum-saline, and gas production. Large numbers of organisms were seen microscopically. As the cultures aged, a sediment accumulated in the serum-saline; large amorphous masses of debris appeared on microscopic examination. In the majority of the cultures, motile trophozoites could be found for about eight days after inoculation (Table 1). As the cultures aged, they diminished in number, their cytoplasm contained many vacuoles and phagocytosis was depressed. In cultures no longer showing viable trophozoites, remnants of these were seen as small bits of cytoplasm.

In the tubes to which from 1000 to 3000 units of streptomycin per cc of serum-saline were added, the trophozoites grew readily in most instances. However, the number of amoebae found after 24 and 48 hours was usually less than in the controls and frequently no amoebae could be found for 72 hours in contrast to the heavy growth in 24 hours in the controls. The trophozoites appeared normal in size, motility, and in the ability to phagocytize rice.

We were able to subculture 3 successive generations of trophozoites in tubes containing 1000 units of streptomycin per cc of serum-saline. However, in about

TABLE 1.—*The effect of streptomycin on the survival of cultures of* E. histolytica

Group	Number of cultures	Survival time (days)	
		Average	Range
Controls	30	8.0	4–12
Streptomycin cultures	26	33.7	7–71

one-sixth of the attempts to subculture the strain in this manner, negative cultures were obtained, a circumstance which never occurred in the controls.

A striking feature of the cultures to which streptomycin was added was the longer survival time of active trophozoites as compared to the controls (Table 1). The average survival of viable trophozoites was 33.7 days in contrast to the shorter life span of the controls (8.0 days). In more than half the cultures the trophozoites survived for longer than 40 days and in 9, more than 50 days. In one culture trophozoites were seen after 70 days.

In the tubes containing the streptomycin the bacterial flora was markedly inhibited. In comparison to the controls, the serum-saline was less turbid, there was no pellicle on the surface of the fluid; growth of bacterial colonies on the slant was scant or absent. Fluid aspirated from the tubes contained fewer bacteria.

Cultures from the controls and from the tubes containing streptomycin revealed noteworthy differences. Four organisms could be isolated aerobically from the controls (*B. subtilis, Staphylococcus albus, B. Alcaligenes,* and an unidentified Gram-negative bacillus). The streptomycin tubes contained only a sparse growth of the unidentified bacillus.

COMMENT

Our results indicate that streptomycin in doses ranging from 1000 to 3000 units per cc of serum-saline overlay produced a 400 per cent increase in the length of survival of cultures of trophozoites of *Endamoeba histolytica.* This effect was associated with gross evidence of bacterial inhibition but not with sterilization of the culture. This finding suggests that the relatively rapid disappearance of amoebae

in culture media is due to bacterial overgrowth which produces an environment unfavorable for the survival of the amoebae.

The failures of subcultures of amoeba trophozoites in media containing streptomycin in about 15 per cent of the instances and the relatively slow increase in the number of trophozoites in the streptomycin tubes favors the concept (Balamuth and Howard, 1946) that amoebic growth is stimulated by symbionts and consequently may be inhibited following depression of bacterial growth by streptomycin. Apparently the relation of bacteria to amoebae in cultures is a complex one. On the one hand, bacterial growth makes a medium more favorable for initiating growth of amoebae. However, this is followed by a relatively quick depression of amoebic growth which can be prevented, as we have shown, by streptomycin. These considerations indicate that bacteria are of fundamental importance in the maintenance of the survival of trophozoites and suggest that antibiotic agents which upset this natural relationship may prove of value in the study of the growth of *E. histolytica* in vitro.

SUMMARY

The addition of streptomycin (1000 to 3000 units per cc) prolonged the survival of cultures of trophozoites of *E. histolytica* from an average of 8.0 days to 33.7 days and eliminated the necessity of frequent transplants to maintain the strain. This effect was associated with evidence of bacterial inhibition but not with sterilization of the culture.

The authors are indebted to Dr. Gregory Shwartzman for his interest in our experiments and his helpful guidance.

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A TECHNIQUE FOR THE PERFUSION OF LABORATORY ANIMALS FOR THE RECOVERY OF SCHISTOSOMES*

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The search for and the recovery of schistosomes from laboratory animals presents a problem if the total number of worms in the animal must be ascertained, or if comparative figures must be compiled on the number of worms present in the blood vessels of various organs and regions. The most important area involved is the mesenteric-portal system, but worms may also be present, especially during their migration, in the pulmonary and systemic circulations. Inspection of the mesenteric veins and main portal vein, and manual dissection or comminution of the liver are not likely to reveal all of the worms, especially the small ones, and the time consumed in removing them individually is considerable. A search for worms early in the infection would be impossible by this technique. A desirable method is one which will standardize the individual differences of the operators, effect a reliable removal of worms, and be applicable to early infections.

A modification of the perfusion technique of Faust and Meleney (1924) serves these purposes. The principle of this technique is to isolate the organs and regions to be examined and to perfuse the vessels in which the worms may be present from their smallest to their largest branches. They accomplished this in the case of the mesenteric system and the extremities by perfusing from artery to vein, in the case of the liver by perfusing from hepatic vein to the portal vein, and in the case of the lungs by perfusing from artery to vein.

Improvements of the technique, made in this laboratory, include supplying a continuous flow of the perfusing fluid, arranging the apparatus in such a way that several animals may be perfused simultaneously, and adding details to obtain more complete collection of worms.

APPARATUS

The apparatus is set up as follows (Fig. 1): A 5-gallon carboy of citrated saline (0.75% sodium citrate and 0.85% sodium chloride in distilled water) is fitted with a three-holed rubber stopper. Pressure tubing is used throughout. Compressed air is fed to the carboy through a heavy Pyrex filter flask used as a trap. The air is conducted to the bottom of the carboy through rubber tubing so that the rate of the air bubbling through the citrated saline may be observed and regulated. One of the two remaining holes in the rubber stopper is for an escape valve, consisting of a short piece of glass tubing to which is attached a piece of rubber tubing with a screw clamp. The third hole is for the delivery system. This consists of glass tubing inside the carboy and of rubber tubing outside running the length of the

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work table. It has a number of glass T connections inserted along its length for outlet lines.

Both stoppers and all connections must be wired securely to withstand the pressure. Each outlet line is provided with a Mohr pinch-clamp near its terminal end and terminates either in a glass cannula with a bulb near the tip, or in an adapter of the "Luer-Lok" type. The cannulae are used for the larger animals such as rabbits and dogs. For smaller animals hypodermic needles of various gauges can be inserted into the adapters depending upon the size of the animal to be perfused. A 22-gauge needle for mice, a 20-gauge needle for hamsters, and an 18-gauge needle or a glass cannula for guinea pigs are recommended.

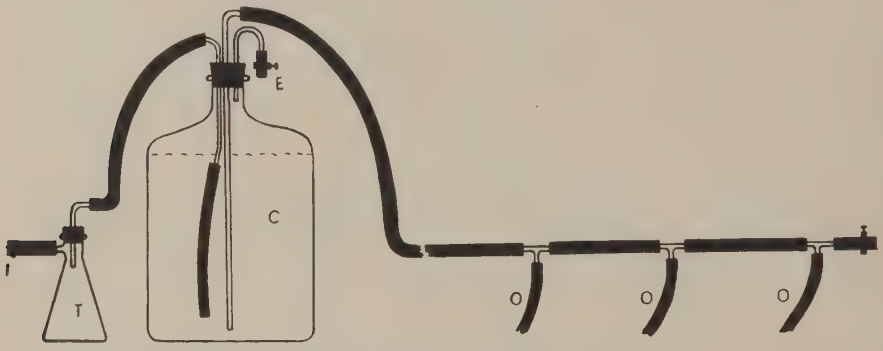

FIG. 1. Apparatus for perfusing several animals simultaneously under positive air pressure. I—air intake; T—trap; E—escape valve; C—carboy; O—outlet.

PROCEDURE

The animal is killed by ether, is fastened to an autopsy board, and is opened by ventral incision. Perfusion should be started before the blood in the animal coagulates.

Perfusion of Liver

In each case where a vessel is doubly ligated, enough space should be left between the ligatures to permit severing the vessel. The inferior vena cava is doubly ligated and severed in each of two places, namely above the diaphragm (Figs. 2, 5) and between the entrance of the right renal vein and the liver (Figs. 3, 6). The portal vein is doubly ligated and severed close to its entrance to the liver (Fig. 3). Care must be taken not to trap any worms between these ligatures. It is advisable to leave a long tab of thread on the portal ligatures on both sides of the cut (Fig. 7). This facilitates locating the portal vein after the liver has been removed. The esophagus should be severed either above the diaphragm or near its entrance into the stomach. In larger animals, it is advisable to use a single ligature on the stomach side of the cut to prevent a backflow of the stomach contents when the mesenteric vessels are being perfused.

The liver can now be removed without loss of intra-hepatic blood. In freeing the liver it is advisable to cut the diaphragm as close as possible to the body wall on all sides so that the liver can be held by the diaphragm while it is being perfused. At the dorsal attachment to the diaphragm, care must be exercised in cutting the liver free so that the aorta is not cut and the liver tissue is not cut or damaged. When

the liver is free it is picked up by lifting the diaphragm and is transferred to a suitable container. A 4-inch Petri dish is adequate for a mouse liver, a 4-inch crystallizing dish for a hamster liver, a pan or a liter beaker for a rabbit liver. The portal ligature, easily located by the long tab, is cut. Lifting the ventral edge of the diaphragm reveals the hepatic vein as a large sinus between the diaphragm and the liver. The cannula or needle on the outlet line is then inserted into the hepatic vein toward the liver (Figs. 4, 8), the pinch-clamp is opened, and the fluid, under pressure, flows through the hepatic veins into the portal system, reaching the exit at the portal vein. By changing the angle of the needle the stream of fluid can be directed into the branches of the hepatic vein from each lobe. The liver expands

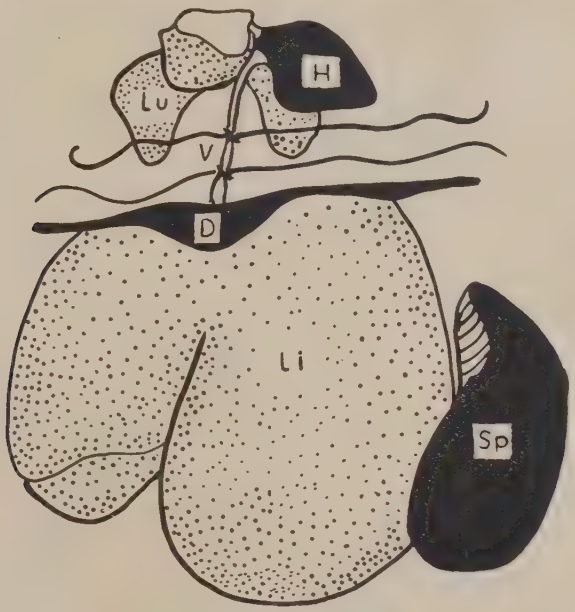

FIG. 2. Double ligation of the vena cava above the diaphragm. H—heart; Lu—lung; V—vena cava; D—diaphragm; Li—liver; Sp—spleen.

to about twice its normal size and is soon washed free of blood and worms. Perfusion is continued until the fluid leaving the organ is free of blood and the tissues are completely blanched. When perfusion is completed, the liver is comminuted in citrated saline. After standing for some time this material is examined for any worms which may have remained trapped in the liver.

In larger animals, such as the rabbit, it is advantageous to allow the liver to remain in situ until all other perfusions are completed. In this case the two pairs of ligatures on the vena cava and a single ligature on the portal vein close to the liver must be applied before any perfusion is attempted. It will be found that during mesenteric perfusion through the aorta as described below, the liver, fed by the small hepatic artery, will become somewhat engorged with the perfusing fluid, but since the other vessels around the liver are ligated there will be no loss of blood or fluid. If the liver is removed after the other perfusions are completed it may be

accomplished more freely and quickly since damage to surrounding vessels and tissues is no longer of any consequence.

Perfusion of Mesenteric Vessels

For small animals (mice, hamsters, etc.).—A single ligature should be applied to the thoracic aorta below the aortic arch to prevent backflow of perfusing fluid, and the fat should be stripped from the surface of the aorta. Then the remaining ligature on the portal vein is cut. A needle is inserted into the thoracic aorta below its ligature. The animal is supported in the left hand with the lower extremities pointing downward so that fluid will flow into the collecting container. The container is

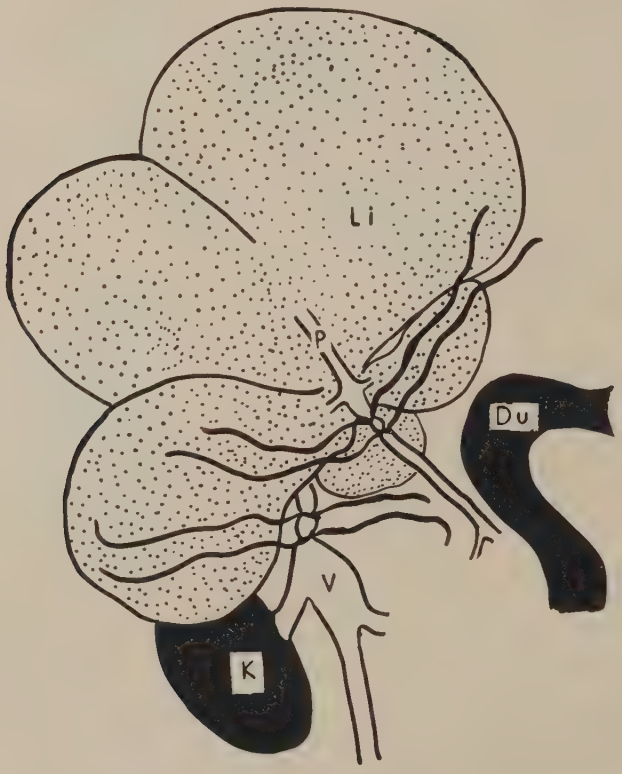

FIG. 3. Double ligation of the portal vein and the inferior vena cava. Li—liver; P—portal vein; K—right kidney; V—inferior vena cava; Du—duodenum.

of the same size as recommended for the liver perfusate. The needle is guided by the right hand. The citrated saline under pressure, controlled by the pinch-clamp, is then allowed to flow into the aorta. The blood and worms in the superior and inferior mesenteric vessel flow out from the portal vein. The vessels become glistening and colorless. Coils of intestine which do not blanch at first should be manipulated gently to relieve kinking of the vessels. After perfusion, the viscera should be washed with a strong stream of citrated saline to remove any worms which may have remained on them after being washed from the vessels. As an additional precaution an

inspection of the blanched vessels should be made for worms remaining in them. Occasionally one or two such worms may be found.

For large animals (rabbit, dog).—The animal should remain tied to the autopsy board or table. The lungs are reflected to expose the thoracic aorta and a ligature is applied just below the arch. An artery clamp is applied to the aorta caudad to the point where the cannula is to be inserted. The wall of the aorta is held with forceps and a transverse incision, large enough to admit the cannula, is made with fine scissors. The cannula, attached to an outlet line, is tied into the aorta, and the artery clamp is removed. It is advisable to include in this ligation the azygos vein, which runs parallel to the aorta, in order to eliminate a source of bleeding during

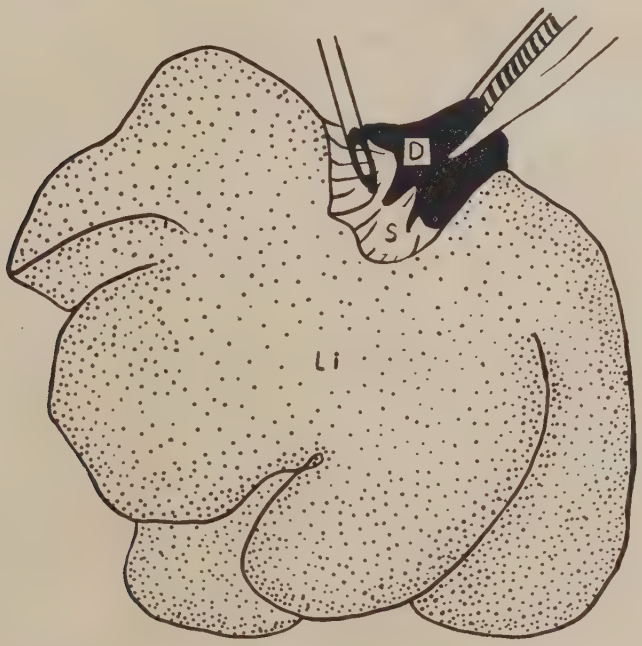

FIG. 4. Hypodermic needle inserted into hepatic sinus for perfusion of liver. D—diaphragm; S—sinus; Li—liver.

perfusion. An artery clamp is placed on the portal vein on the mesenteric side of its single ligature, leaving sufficient space to allow a cannula to be inserted between it and the ligature. If worms are present in this portion of the vessel they should be pushed gently toward the intestine. The cannula is prepared by attaching a length of rubber tubing, filling it with citrated saline, and clamping the tubing with a hemostat in order to retain the fluid in the cannula. This is essential to prevent clot formation in the vessel or in the tubing. An incision is made in the vessel, the cannula is inserted toward the mesenteric veins and is tied in place.

Care must be taken that the tip of the cannula does not extend beyond the branching of the superior mesenteric vein thereby obstructing the flow from one of the branches. The artery clamp is then removed from the portal vein and some of the solution in the cannula is forced into the vessel by compressing the rubber tubing.

The free end of the rubber tubing is placed in an appropriately labeled one-liter beaker or flask and the pinch-clamp is removed from it. Perfusion is then performed as in smaller animals.

Perfusion of Other Regions

For some experiments such as tracing the route of migration of young worms and recovering adult worms which may be in unusual situations, perfusion of the pulmonary or systemic circulations may be necessary. Because of their limited application it seems inappropriate to describe in this paper the technique which has been developed for these purposes. It will be described in a subsequent paper dealing with the migration of young worms from the skin and peritoneal cavity to their adult locations.

Recovery of Worms from Perfusates

The bloody perfusates can be laked with water if only the worm count is needed. A large volume of water is added and the worms allowed to settle in the container for about a half hour. The supernatant fluid is then aspirated carefully with a filter pump to within about ¾ inch of the bottom of the container. If the overlying fluid is poured off care must be taken that none of the sediment escapes. The sediment is poured into glass evaporating dishes and examined for worms under a binocular dissecting microscope. Large worms are easily lifted out of the solution with an applicator stick tapered at one end. For small worms a capillary pipette must be used.

If the worms are to be preserved the perfusate must not be laked because in hypotonic solutions they become bloated and die. In this case the perfusate should be allowed to sediment. When the supernate is clear, Petri dishes can be put under the dissecting microscope and the worms may be seen standing out in sharp relief against the background of erythrocytes. In larger containers, after sedimentation has proceeded for a half-hour the supernate is aspirated and the sediment is poured into glass evaporating dishes. By rotating the dishes rapidly the worms and some of the cells are immediately pulled to the center of the dish. The supernate is drawn off with a pipette and fresh physiological saline is added. Rotation of the dish, removal of the supernate and addition of new saline are repeated until the fluid is clear enough for the worms to be visible through it under the dissecting microscope.

DISCUSSION

The above technique has been described in detail because our experience has shown that the procedures used by most recent investigators in connection with worm recovery in chemotherapeutic studies fail to recover a considerable proportion of the worms. We believe that more accurate estimations of the effect of chemotherapeutic agents can be made if the technique of recovery can be standardized at a degree of efficiency which will reveal practically the entire worm burden. When the above technique is mastered it requires practically no more time than the less efficient methods. For instance a skilled technician can complete the perfusion of a mouse in fifteen minutes. The time required for counting the worms depends, of course, upon the worm burden and their stage of development.

Brandt and Finch (1946) recommended heparin to prevent coagulation of the blood in recovering schistosomes from rabbits. The heparin (100 mg in 30–40 ml of physiological saline) was injected intravenously or intracardially 15–30 minutes

before sacrificing the animal. The liver was drained of as much blood as possible by aspirating through an 18-gauge needle from the portal vein, after which the portal and hepatic veins were ligated, the liver removed, its margins slit, the organ perfused through the hepatic vein, and finally teased apart. The heparinized blood was poured into a 0.5 per cent solution of saponin in physiological saline to lake the erythrocytes. The authors stated that this "causes no apparent damage to the worms".

Heparin is preferable to sodium citrate as an anticoagulant if the metabolism of schistosomes is to be studied, since sodium citrate interferes with their oxygen uptake and glycolysis. Bueding, in a personal communication, states that a concentration of 3×10^{-2} molar (0.88%) almost completely inhibits these metabolic processes, and that a 3×10^{-3} molar concentration inhibits glycolysis slightly. However, if the worms are to be collected for other purposes than metabolic studies, citrate appears to cause no damage, since, if the worms are transferred to physiological saline shortly after removal from the host, they will survive for several hours at room temperature and for several days at 4° C. Since the cost of heparinizing a rabbit is about twenty times the cost of the sodium citrate used in perfusing a rabbit by our technique, it seems inadvisable to recommend heparin if a large number of animals is to be used.

The observation of Brandt and Finch on the use of saponin suggested the possibility of substituting it for sodium citrate in the perfusing fluid in order to lake the blood, but the attempt was unsuccessful. In six mice in which a 0.5 per cent solution of saponin in physiological saline was used to perfuse the mesenteric veins through the aorta the leg muscles of the mice became rigid and the worms remained in the mesenteric veins, although the blood was laked. When the worms were removed from the veins with needles, and placed in saponin-saline, they were at first hyperactive but soon coiled up tightly and became motionless. It was not possible to relax them for proper fixation.

We believe, therefore, that perfusion with citrate-saline using the technique we have described, is at present the preferred method of recovering the maximum proportion of worms for most purposes in the study of schistosomiasis in experimental animals.

SUMMARY

A technique is described for the perfusion of the mesenteric-portal system in recovering schistosomes from experimental animals. The apparatus provides the opportunity of perfusing several animals simultaneously. Citrated saline is considered the most practical perfusing fluid unless the worms are to be used alive for metabolic studies. It is believed that the technique described will recover practically all of the worms regardless of their stage of development, and without requiring more time than by less accurate techniques. It is also believed that the accurate estimate of the worm burden obtainable by this technique will produce more conclusive results than have been obtained by the use of less refined techniques in estimating the effect of chemotherapeutic agents on the worms.

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Fig. 5

Fig. 6

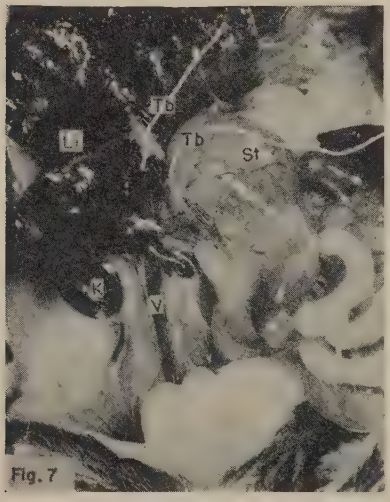

Fig. 7

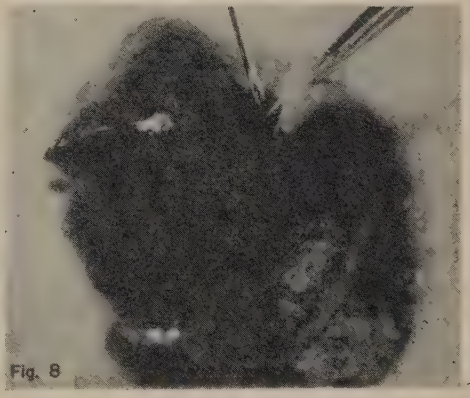
Fig. 8

EXPLANATION OF PLATE

(Photography by Mr. S. F. Yolles)

ABBREVIATIONS USED

D—diaphragm
Du—duodenum
H—heart
K—right kidney
Li—liver
Lu—lung
S—sinus
SP—spleen
St—stomach
Tb—thread tabs
V—inferior vena cava

FIG. 5. Cutting vena cava between the ligatures above the diaphragm (mouse).

FIG. 6. Double ligature on inferior vena cava between the entrance of the right venal vein and the liver (mouse).

FIG. 7. Appearance of under surface of liver after the portal vein and inferior vena cava have been cut between the ligatures. Liver is now ready for removal from body of mouse. Long thread tabs identify cut ends of portal vein.

FIG. 8. Mouse liver ready for perfusion.

A NEW SPECIES OF *RENIFER* (TREMATODA) FROM THE KINGSNAKE, *LAMPROPELTIS GETULUS*, WITH AN EMENDATION OF THE GENUS *RENIFER* PRATT, 1903

IRVING G. KAGAN[1]

From the trachea, esophagus and mouth of a kingsnake, *Lampropeltis getulus floridana* Blanchard, approximately 140 trematodes belonging to a single species of *Renifer*, as yet undescribed, were recovered at autopsy and fixed in Bouin's fluid. The host was purchased from a dealer in Florida and died several weeks after arrival in the laboratory. My material was received from Mr. Colvin L. Gibson, who observed at autopsy that the entrance to the trachea was completely occluded by a mass of flukes herein described. This study is based on more than 50 mature adults and the specific diagnosis is made from 12 gravid adults fixed and mounted in toto and from sections of several mature worms. Whole mounts were stained with Reynold's mixture (Delafield's haematoxylin and alum cochineal), Delafield's haematoxylin, or Semichon's acetocarmine. Sections were stained in iron haematoxylin.

In the diagnosis which follows, measurements of central tendency are stated in terms of the mean and its standard error. The mean in nature has a 68.26% chance of falling within a range of plus or minus one standard error from the computed mean; a 95.44% chance within a range of plus or minus 2 times the standard error; a 99.74% chance within a range of 3 times the standard error. In computation of the standard error the number of degrees of freedom were taken into account.

Renifer floridanus sp. nov.

Specific diagnosis: Characters of genus. Body elongate, sides almost parallel, ends rounded. Somewhat flattened ventrally, arched dorsally; length 3.72 ± 0.181 mm, width 1.105 ± 0.174 mm. Cuticula spinose. Oral sucker subterminal, 0.318 ± 0.010 mm long by 0.316 ± 0.011 mm wide. Acetabulum larger than oral sucker, 0.503 ± 0.029 mm long by 0.567 ± 0.019 mm wide. Prepharynx short. Pharynx muscular 0.114 ± 0.008 mm long by 0.146 ± 0.011 mm wide with gland cells. Esophagus 0.219 ± 0.023 mm long. Ceca narrow and tubular, 0.069 ± 0.006 mm wide, extending slightly beyond acetabulum. Genital pore at left margin at level of pharynx. Cirrus sac muscular, 0.788 ± 0.051 mm long and 0.108 ± 0.006 mm wide, extending diagonally, slightly beyond midline of body to anterior margin of acetabulum. Vesicula seminalis internal and coiled, pars prostatica long, prostate cells numerous, ductus ejaculatoris long, somewhat inflated, cirrus slender and unarmed. Ovary ovoidal, 0.146 ± 0.010 mm long by 0.132 ± 0.013 mm wide, lying at the posterior lateral margin of acetabulum. Mehlis' gland and Laurer's canal present. Receptaculum seminis absent. Testes somewhat lobate, opposite. Right testis 0.339 ± 0.027 mm long by 0.210 ± 0.022 mm wide. Left testis 0.315 ± 0.020 mm long by 0.224 ± 0.017 mm wide. Vas deferens lacking. Uterus sinuous, extending to hinder part of body; descending limb slender, upper part filled with semen and serving as a seminal receptacle; ascending limb broad. Metraterm adherent to cirrus sac, musculature weak. Vitellaria follicular, averaging 50 follicles per side, marginal, in middle third of body, ending slightly posterior to testes. Eggs small, numerous, operculate, 0.0335 mm long by 0.0167 mm wide.

Host: Lampropeltis getulus floridana Blanchard.

Habitat: Trachea, esophagus, and mouth.

Locality: Unknown, probably central and southern Florida.

Type specimen: Slide no. 45795, and paratype no. 45796, U. S. National Museum.

This species is distinct from the other species of the genus, but shows close affinity to *Renifer kansensis* Crow 1913, from *Ancistrodon contortrix*, *R. septicus*

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[1] A contribution from the Department of Zoology, University of Michigan, prepared under the direction of George R. LaRue.

MacCallum 1921, and *R. ophiboli* MacCallum 1921, both from *Lampropeltis getulus*. *Renifer floridanus* n. sp. differs from *R. kansensis* in the presence of a Laurer's canal, a larger acetabulum, smaller oral sucker, pharynx, esophagus, testes, ovary, and egg. It differs from *R. septicus* in the more anterior position of the genital pore, the smaller ovary and testes and egg, the shorter ceca and larger cuticular spines; and from *R. ophiboli* in the more anterior position of the genital pore, the larger size ratio of oral sucker to acetabulum, the smaller cirrus sac, vesicula seminalis, and egg.

MacCallum's description of *R. septicus* was inaccurate in several respects. An examination of his original material borrowed from the U. S. National Museum shows the intestinal ceca overlapping the testes and not as short as he figured them; the anterior third of the descending limb of the uterus actually lies dorsal to the ascending limb; a Mehlis' gland is present; the cuticula finely spinose. The testes in his figure show outlines of the shrunken testicular contents and not of the tunica. These differences may be explained by the fact that his drawings probably were made by an artist not familiar with the material. To correct these inaccuracies, *R. septicus* is herein recharacterized from the original material consisting of one slide containing two whole and one broken specimens.

Renifer septicus MacCallum, 1921, emend.

Specific diagnosis: Characters of genus. Body elongate, sides parallel, ends rounded. Cuticular spines small, closely set. Length 3.4 (3.3–3.5) mm, width 1.0 mm. Oral sucker subterminal 0.285 mm long by 0.321 (0.304–0.342) mm wide. Acetabulum larger than oral sucker, 0.367 (0.304–0.399) mm long by 0.437 (0.418–0.456) mm wide. Prepharynx present. Pharynx muscular, 0.154 (0.140–0.177) mm long by 0.164 (0.136–0.192) mm wide with gland cells. Esophagus 0.268 (0.158–0.342) mm long by 0.042 (0.40–0.45) mm wide. Ceca 0.145 (0.117–0.209) mm wide overlap testes. Genital pore lateral midway between bifurcation of intestinal ceca and pharynx. Cirrus sac slender and muscular, 0.896 (0.836–0.970) mm long, extending beyond midline of body to middle of acetabulum. Vesicula seminalis large, internal, and coiled, pars prostatica long, prostate cells numerous, cirrus slender. Ovary oval, 0.184 (0.167–0.203) mm long by 0.216 (0.194–0.217) mm wide, overlaps posterior lateral margin of acetabulum. Mehlis' gland present. Laurer's canal not observed. Receptaculum seminis absent. Testes large lobate and opposite. Right testis 0.475 (0.473–0.477) mm long by 0.375 (0.323–0.437) mm wide. Left testis 0.458 (0.448–0.513) mm long by 0.351 (0.323–0.380) mm wide. Vas efferentia not observed. Uterus narrow, descending limb straight, extending to hind part of body where it bends sharply forward; ascending limb straight. Metraterm adherent to cirrus sac. Musculature weak. Vitellaria lateral, in middle third of body, extending slightly beyond testes. Eggs operculate 0.037 mm long by 0.019 mm wide.

Host: Lampropeltis getulus.

Habitat: "Under the scales of the skin."

Type specimen: Slide no. 36353, U. S. National Museum.

In my opinion the type and cotype specimens of *R. septicus* are not fully developed. Conflicting data as to the rate of maturing have been secured in the life cycles studied within this subfamily. Byrd (1935) reported the recovery of young specimens of *Renifer aniarum* (Leidy 1890), from the mouth cavity of the water snake, *Natrix sipedon fasciata* within 28 days after initial feeding with infected tadpoles, and mature flukes within 35 days. Talbot (1933) reported that *Lechriorchis primus* (Stafford 1905) reached the lungs of the definitive host, *Thamnophis sirtalis*, 10 to 12 months after feeding and then after reaching the lungs do not become sexually mature until the following summer, the maximum size probably not being attained until the third year. Ingles (1933) did not complete the life cycle of *Zeugorchis syntomentera* Sumwalt 1926 experimentally and did not contribute data on the developmental stages of the young trematodes. The presence in my collection of many small mature worms and relatively few worms with completely

distended uterus strongly indicates a somewhat prolonged period of growth after attainment of sexual maturity as Talbot pointed out. A representative series shows a gradual transition from the simple looped uterus of the smaller worms to the serpentine, distended uterus of the larger gravid worms. The simple looped uterus of MacCallum's specimens of *R. septicus* indicates that the specimens have not reached their maximum growth and development.

Schmidt and Hubbard (1940) reported *Renifer serpentis* from *Agkistrodon piscivorus* as provisionally new, stating that "the anatomical differences between the two forms [i.e., their species and *Renifer septicus*] are perhaps too slight to be significant." None of their specimens deposited in the U. S. National Museum are fully developed. However, since the original material of *R. septicus* MacCallum 1921 is also not fully developed a valid comparison can be made. The larger size of body, testes and vesicula seminalis, the spinose cuticula, longer intestinal ceca and smaller egg distinguish *Renifer septicus* from *Renifer serpentis*.

Byrd and Denton (1938) considered that *R. ophiboli* MacCallum 1921 differed from *R. septicus* MacCallum 1921 in the more spent condition of the vitellaria, the shrunken testes and ovary, and failure of the cirrus sac to enclose the vesicula seminalis. They further stated, "When we take into account the full development of the internal anatomy of *R. septicus* and the senescent condition exhibited by *R. ophiboli* we feel justified in considering the two species to be identical." However a comparison of MacCallum's type specimen of *R. ophiboli* with his published drawing shows the vesicula seminalis enclosed in the cirrus sac, the cuticula completely spinose, the ascending limb of the uterus in his figure to be actually the descending limb, and the genital pore at the level of the bifurcation of the intestinal ceca instead of anterior to the bifurcation. MacCallum's original material consisted of one type specimen only. An examination of the type specimen leads me to believe that it is not a senescent worm. The ascending limb of the uterus, except for a few eggs, is barren. One may conjecture that the worm had been left in a hypotonic solution causing the uterus to swell and expel its eggs. The position of the genital pore, the possession of long cuticular spines, the smaller ratio of oral sucker to acetabulum, smaller ovary and testes and larger egg distinguish *R. ophiboli* from *R. septicus*.

Renifer ophiboli MacCallum, 1921, emend.

Specific diagnosis: Characters of genus. Body elongate, sides parallel, anterior end rounded, posterior end slightly pointed. Cuticula spinose. Length 5.1 mm, width 1.34 mm. Oral sucker subterminal, 0.437 mm long by 0.437 mm wide. Acetabulum larger than oral sucker, 0.532 mm long by 0.551 mm wide. Prepharynx present. Pharynx muscular, 0.167 mm long by 0.186 mm wide, gland cells numerous. Esophagus 0.399 mm long. Ceca narrow, ends bulbus, terminating short of testes. Genital pore at level of bifurcation of intestinal ceca. Cirrus sac muscular, 1.04 mm long by 0.192 mm wide, extending beyond midline of body to anterior margin of acetabulum. Vesicula seminalis large, internal, coiled, pars prostatica short, prostate glands numerous, ductus ejaculatorius slender, cirrus unarmed. Ovary small, ovoidal, 0.135 mm long by 0.204 mm wide, lying at posterior lateral margin of acetabulum. Mehlis' gland present, Laurer's canal not observed. Receptaculum seminis lacking. Testes lobate, nearly opposite. Right testis 0.399 mm long by 0.437 mm wide. Left testis 0.418 mm long by 0.380 mm wide. Vas deferens lacking. Uterus sinuous, descending limb narrow, ascending limb broad. Vitellaria follicular, lateral, in middle third of body extending slightly behind testes. Eggs operculate, 0.045 mm long by 0.022 mm wide.

Host: Lampropeltis getulus.

Habitat: Large intestine.

Type specimen: Slide no. 36362, U. S. National Museum.

The position of the genital pore in *R. septicus* makes it desirable to reconsider

in detail the generic diagnoses of the genera *Renifer* Pratt 1902[2] and *Neorenifer* Byrd and Denton 1938. The genus *Renifer* was emended by Byrd and Denton to read, "Genital pore lateral outside area between bifurcation of ceca and acetabulum on level with bifurcation of ceca." For the genus *Neorenifer* they stated, "Genital pore to one side of midline, on level with pharynx or oral sucker." The genital pore of *R. septicus* lies midway between bifurcation of ceca and pharynx and therefore between the two designated areas and fits neither diagnosis. If their ideas are to be followed to the logical conclusion it would be necessary to establish a third genus to accommodate species with the genital pore midway between pharynx and bifurcation of ceca. A third genus is not fitting or necessary. The position of the genital pore shows considerable variability in my specimens. In four gravid specimens the genital pore is slightly posterior to the pharynx and in eight at the level of the pharynx. Allison and Holl (1937), in their description of *Renifer brachyoesophagidius,* stated, "The genital pore is situated lateral and just anterior to intestinal bifurcation, although in some specimens, the genital pore is at the same level as intestinal bifurcation." MacCallum's figure of *R. ophiboli* shows the genital pore anterior to bifurcation of ceca. This misrepresentation led Byrd and Denton to place the species in their genus *Neorenifer.* However the genital pore is at the level of the bifurcation of ceca and the species would thus fall into the genus *Renifer* as emended by them.

The second character used by Byrd and Denton to separate the genus *Neorenifer* from *Renifer* is the possession of a "rather weakly developed cirrus sac." A comparison of cirrus sacs of the species *ophiboli, septicus* and *floridanus* shows no marked difference in development. This character thus fails to separate the species into two genera. In light of the variability of the position of the genital pore, and the fact that there is little difference in the development of the cirrus sac in these worms, it would be better to redefine the genus *Renifer* to include all the species whose genital pores are lateral, extracecal, and lying between bifurcation of ceca and oral sucker. It is therefore proposed that the genus *Renifer* be so emended as to include the species of the genus *Neorenifer.*

Genus *Renifer* Pratt, 1903, emend.

Generic diagnosis: Characters of the subfamily. Body elliptical. Cuticula with or without spines. Acetabulum slightly larger than oral sucker. Ceca variable in length, sometimes directed toward center of body between testes. Genital pore lateral, extracecal, between level of bifurcation of ceca and oral sucker. Metraterm weak to well developed, adherent to or near cirrus sac. Esophagus short or absent. Cirrus pouch not extending posterior to acetabulum. Vitellaria lateral, in central third of body, sometimes divided into two groups. Parasitic in digestive, respiratory, and reproductive tract of snakes.

Type species: Renifer ellipticus Pratt 1903.

Additional species.—R. ancistrodontis MacCallum 1921, *R. brachyoesophagidius* (Allison and Holl 1937), *R. floridanus* sp. nov., *R. grandispinus* Caballero 1938 (syn. *N. drymarchon* Byrd and Denton 1938), *R. laterotrema* Byrd and Denton 1938, *R. magnus* Byrd and Denton 1938, *R. megametricus* (Talbot 1934), *R.* n. sp. Job 1917. The following species formerly assigned to the genus *Neorenifer* Byrd and Denton 1938 are returned to the genus *Renifer: R. acetabularis* Crow 1913,

[2] The name *Renifer* first appeared in print in 1902 in Pratt's "Synopses of North-American Invertebrates" XII. The Trematodes, Amer. Nat. **36**: 888, 899, 957, where it appeared in a key to the genera of trematodes, but without formal description or designation of type species. The formal generic description was first published in Pratt's "Description of Four Distomes," Mark Anniv. Vol., 1903.

R. aniarum (Leidy 1890) (syn. *R. natricis* MacCallum 1921, *R. texanus* Harwood 1932), *R. elongatus* Pratt 1903, *R. formosum* (Nicoll 1911), *R. kansensis* Crow 1913, *R. ophiboli* MacCallum 1921, *R. orula* Talbot 1934, *R. sauromates* (Poirier 1885), *R. septicus* MacCallum 1921, *R. validus* (Nicoll 1911) (syn. *Lechriorchis inermis* Lebour 1913), *R. wardi* Byrd 1936, *R. zschokkei* (Volz 1899). The following species which were originally described as belonging to the genus *Neorenifer* are now placed in the genus *Renifer: R. georgianus* (Byrd and Denton 1938) comb. nov., *R. glandularis* (Byrd and Denton 1938) comb. nov., *R. heterodontis* (Byrd and Denton 1938) comb. nov., *R. serpentis* (Schmidt and Hubbard 1940) comb. nov.

SUMMARY

1. *Renifer floridanus,* new species, is described and proposed as a new member of the genus *Renifer.*

2. *Renifer septicus* MacCallum 1921 and *Renifer ophiboli* MacCallum 1921 are shown to be distinct species and are recharacterized.

3. *Renifer serpentis* (Schmidt and Hubbard 1940) is validated as a species of the genus *Renifer.*

4. The genus *Renifer* Pratt 1903 and the genus *Neorenifer* Byrd and Denton 1938 are shown not to be distinct and the genus *Renifer* Pratt 1903 is emended to include the species of the genus *Neorenifer* Byrd and Denton 1938.

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EXPLANATION OF PLATE

All drawings are drawn to scale with the aid of a microprojector or camera lucida.

ABBREVIATIONS

B ..... Bladder	UP ..... Uterine passage
LC ..... Laurer's Canal	VD ..... Vitelline duct
OÖt ..... Oötype	VR ..... Vitelline reservoir

FIG. 1. Cross-section through posterior portion of acetabulum showing uterus, Laurer's canal, ovary, intestinal ceca, and vitellaria.

FIG. 2. Composite drawing of a portion of female reproductive system from cross-sections.

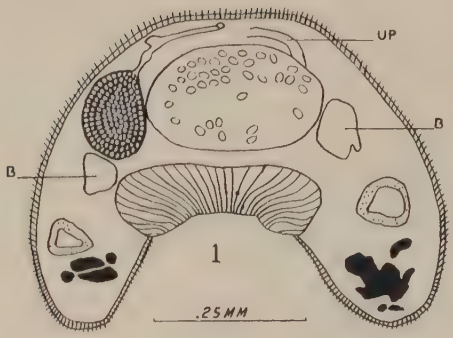
FIG. 3. Section of cuticle with spines from middle of dorsum.

FIG. 4. Outline drawings of eggs from the anterior portion of uterus of whole mounts. Left to right eggs of *R. ophiboli, R. septicus,* and *R. floridanus,* drawn to same scale.

FIG. 5. *Renifer ophiboli* MacCallum 1921. Ventral view of type specimen.

FIG. 6. *Renifer septicus* MacCallum 1921. Ventral view of type specimen.

FIG. 7. *Renifer floridanus* sp. nov. Ventral view. The eggs in figures 5, 6, and 7 are conventionalized and not representative of correct size and shape.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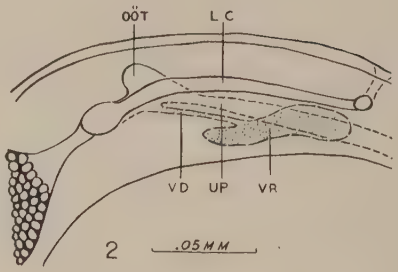
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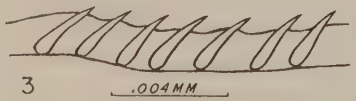
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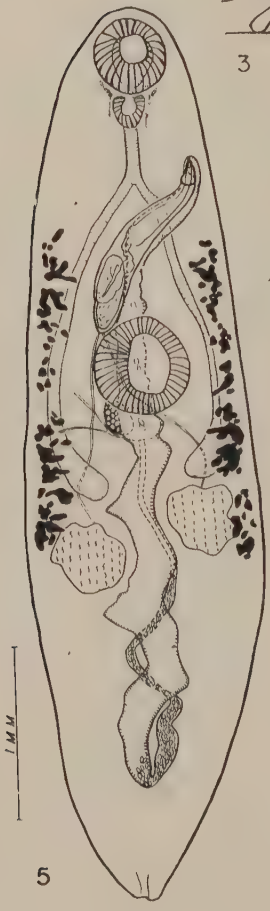
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OBSERVATIONS ON THE RÔLE OF *TENEBRIO MOLITOR* AS AN INTERMEDIATE HOST FOR *HYMENOLEPIS NANA* VAR. *FRATERNA*

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Bacigalupo (1928a, 1928b, 1928c, 1929) was the first to report the successful infection of the grain beetle, *Tenebrio molitor,* and other insects with the cysticercoids of *Hymenolepis nana* var. *fraterna*. He suggested that in Argentina the usual life cycle is indirect, involving an intermediate host. Otto (1936) stated, "However, utilization of an intermediate host by this form (*H. nana*) in the United States has never been demonstrated and Joyeux and Baer (1932) have shown that it is very uncommon in Europe."

As there has as yet been no verification of Bacigalupo's work in this country, the writer feels it advisable to present a report of the successful infection of *T. molitor* with *H. nana* var. *fraterna* from mice.

The grain beetles (*T. molitor*) were obtained from a colony kept in the Dept. of Zoology and Entomology of the Alabama Polytechnic Institute. During the course of the experiment they were kept in glass jars containing wheat bran. They were determined to be previously uninfected by the examination of a small control group along with the first group of beetles infected with *H. nana* and by the failure to find cysticercoids in infected beetles until sufficient time had elapsed for their development.

In the first test, 5 *T. molitor* were fed gravid segments from one specimen of *H. nana* obtained from a wild mouse, *Mus musculus*. No cysticercoids were found in a beetle examined 4 days later, nor in 2 examined 5 days later, but 2 beetles examined 12 days later had 31 and 10 cysticercoids respectively. One uninfected beetle examined on the 5th day and another examined on the 12th day were negative. Cysticercoids found in the beetles above were fed to a white mouse which was killed 12 days later. Two strobila of *H. nana,* one approximately 95 mm long, were recovered. Fecal samples had been negative.

In the second test, 12 *T. molitor* were fed gravid proglottids from worms in an infected white mouse sent by Dr. John E. Larsh of the University of North Carolina. Eggs from these worms were also fed to a previously uninfected mouse with a resulting infection demonstrating direct transmission. One beetle was examined on the 7th and another on the 9th day after infection; both were negative as were two examined on the 12th day. One of six examined on the 14th day harbored 2 cysticercoids. One of two examined on the 16th day harbored 15 cysticercoids. Ten of these cysticercoids were fed to a white mouse. Fecal samples were positive for onchospheres 14 days later, and necropsy on the 15th day after infection revealed 4 specimens of *H. nana* in the mid portion of the small intestine. These worms measured 80–90 mm in length. Gravid proglottids from these worms were in turn fed to 5 *T. molitor* and several cysticercoids were recovered from one of the 5 beetles when examined about 2 weeks after infection.

Cysticercoids recovered from the beetles in these experiments were not studied

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in detail, but measurements showed the total length to vary from 342 to 572 microns. All specimens were armed, bearing from 21 to 25 hooks.

These experiments demonstrate the successful use of the meal beetle *Tenebrio molitor* as an intermediate host of the tapeworm *Hymenolepis nana* var. *fraterna* from mice. Cysticercoids recovered 12 days after infection were proven infective by feeding to mice. The fact that cysticercoids were not discovered in beetles examined up to 9 days after infection but were found 12–14 days after infection indicates the time necessary for the cysticercoids to reach sufficient size to be detected by the methods employed.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. R. O. Christenson (then of the Dept. of Zoology and Entomology, A.P.I.) for his guidance in the work and for verification of the identification of the parasites; to Dr. D. A. Porter, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama, for his suggestions and references to literature; and to Dr. John E. Larsh, School of Public Health, University of North Carolina, for supplying mice infected with *H. nana*.

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BAKERERPES FRAGILIS N. G., N. SP., A CESTODE FROM THE NIGHTHAWK (CESTODA: DILEPIDIDAE)

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The examination of an Eastern Nighthawk (*Chordeiles m. minor* (Forster)), collected September 25, 1945, at Marion, Ohio, revealed numerous, very small cestodes free in the lumen of the small intestine. About fifty specimens were found, but, because of the delicate strobila of this species, few were entire.

Although these cestodes were very thin and translucent, the preparation of well-stained whole mounts presented unusual difficulty. Best results were obtained with a mixture of Ehrlich's and Delafield's haematoxylin stains. Frontal and transverse serial sections, cut at 10 and 15 μ, were made.

This cestode clearly belongs in the sub-family DILEPIDINAE, but, in so far as the writer has been able to determine, the combination of characters is such that it cannot be assigned to any of the existing genera. A new genus has therefore been erected.

Bakererpes n. g.

Diagnosis: Dilepididae. Small, weakly-muscled cestodes, with few segments. Genital pores regularly alternate; genital atrium large, surrounded by large muscular area, and provided with spines. Cirrus sac very large, extending across entire width of mature segments; relatively smaller in other segments. Cirrus spined. Vagina separated into two parts by narrow constriction. Genital ducts pass between longitudinal excretory canals. Rostellum well-developed; armed with few hooks in single row. Testes numerous, posterior and lateral to ovary and vitelline gland. Uterus develops sacculations which later break down, becoming single large sac in gravid segments. Parasites of birds.

Type species: B. fragilis.

Bakererpes fragilis n. sp.

(Figs. 1 to 7)

Diagnosis: Strobila, wedge-shaped, averages 2.5 mm long; greatest width, slightly over 1.0 mm, attained in terminal segment. Usually 8 segments present. Segments broader than long; gradual increase in length to middle of strobila, after which length decreases. Strobila very fragile; musculature extremely weak. Scolex large, averaging 420 μ long by 240 μ wide. Suckers well-developed, about 100 μ in diameter. Rostellum strongly developed, armed with a single row of hooks, 10 in number. Hooks average 80 μ in length.

Excretory canals very small; ventral and transverse canals about 4 μ in diameter; dorsal canal somewhat smaller. Exact course of dorsal canal not determined.

Genital *Anlagen* visible in third segment; transition from immature to gravid segments very rapid. Genital pores regularly alternate; genital ducts pass between longitudinal excretory canals. Genital atrium strongly developed, averaging 90 μ long by 70 μ deep; lined with large (7 μ long) spines, directed medially. Atrium surrounded by large muscular area, about 200 μ long by 150 μ deep; thickness of segment, about 200 μ, greatest through this area.

Cirrus sac very large; greatest size (about 445 by 60 μ) attained in mature segments. Cirrus sac extends to aporal margin in latter; at times aporal end of sac may break through into posterior edge of preceding segment. Ductus ejaculatorius somewhat coiled within cirrus sac; cirrus, about 9 μ in diameter, provided with very numerous, small spines. Internal and external seminal vesicles absent; vas deferens greatly coiled and enlarged before entering cirrus sac. Testes 16 to 20 in number; averaging about 16 μ in diameter when first visible, and reaching a diameter of 36 μ before disappearing in pre-gravid segments. Testes posterior and lateral to ovary and vitelline gland.

Ovary, partially separated into two parts, situated just anterior to center of segment. Ovary enlarges somewhat after segments mature, but disappears before latter become gravid. Vitelline gland, nearly spherical, situated just posterior to ovary, near center of segment.

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Vagina in mature segments takes rather direct course toward margin, and empties into common genital canal just before latter enters genital atrium. Vagina divisible into two parts; a distal section slightly longer than cirrus, into which the latter is introduced, and a proximal section. This division is most obvious in gravid segments. The distal section of the vagina, into which the cirrus is introduced, is larger in diameter than the cirrus, and is separated from the proximal part by a very narrow constriction. The poral end of the proximal section of the vagina enlarges in gravid segments and appears to function as a seminal receptacle, which persists throughout. The cirrus was seen inserted into the vagina only in segments well past maturity.

The early development of the uterus is not clear. It appears to develop numerous, small sacculations which later break down, leaving the eggs free in a single, large sac. The eggs in the specimens available were not fully developed; they measured from 20 to 25 μ in diameter, but had no outer shell.

Host: Chordeiles minor minor (Forster) (Eastern Nighthawk).

Habitat: Small intestine.

Locality: Marion, Ohio.

Type specimen: In the U. S. National Museum Helminthological Collection No. 36995.

DISCUSSION

Of the genera previously assigned to the sub-family DILEPIDINAE, few closely resemble *Bakererpes*. The arrangement of the genital ducts in the latter appears to be unique in this group.

The genus *Liga* Weinland, 1857, resembles the present genus in having regularly alternate genital pores, few segments, and similarly arranged reproductive organs. As with *Bakererpes,* the vas deferens and seminal receptacle persist in the gravid segments in *Liga,* and the form of the uterus is similar. *Liga,* however, has two rows of hooks on the rostellum, and a small cirrus sac, in addition to marked differences in the region of the genital atrium. The eggs of the species of *Liga* are provided with characteristic projections, which may or may not be present on the eggs of *Bakererpes*. The writer had available for comparison specimens of *Liga brasiliensis* (Parona, 1901), from the Flicker. This species was well described by Ransom (1909).

The genus *Amoebotaenia* Cohn, 1899, is similar to the present genus in having a single row of hooks, regularly alternate genital pores, and a somewhat similar arrangement of the genital organs. However, the details of the cirrus sac and the genital pore differ greatly, the testes are arranged in a row in the posterior part of the segment, and the form of the ovary differs from that in *Bakererpes*. The more numerous segments of *Amoebotaenia* are much shorter, with a corresponding arrangement of the genital organs. The writer had available for comparison specimens of *Amoebotaenia* sp. from a warbler, in addition to the descriptions in the literature.

The genus *Krimi* Burt, 1944, has a single row of hooks, approximately the same arrangement of genital organs, and a similar small size, with few segments. *Krimi* differs chiefly, however, in having irregularly alternate genital pores, dissimilar copulatory organs, and a reticulate uterus.

The remaining genera of the sub-family DILEPIDINAE do not resemble *Bakererpes* to a sufficient degree to warrant discussion.

This cestode has been named in honor of Mr. Mark H. Baker, of Marion, Ohio, without whose kind cooperation this work would not have been possible.

The writer wishes to acknowledge information kindly supplied by Dr. E. W. Price from the files of the Bureau of Animal Industry.

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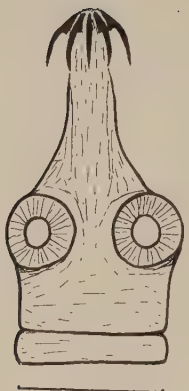
Explanation of Plate

Morphology of *Bakererpes fragilis* n. sp.

Fig. 1. Details of scolex.
Fig. 2. Morphology of an early mature segment.
Fig. 3. Details of a gravid segment (terminal).
Fig. 4. Enlarged hook from the rostellum.
Fig. 5. Entire specimen, showing external appearance and relative size of cirrus sac. Drawn from photomicrograph.
Fig. 6. Details of copulatory organs.
Fig. 7. Details of genital pore.



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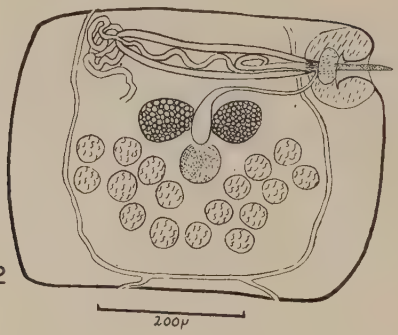

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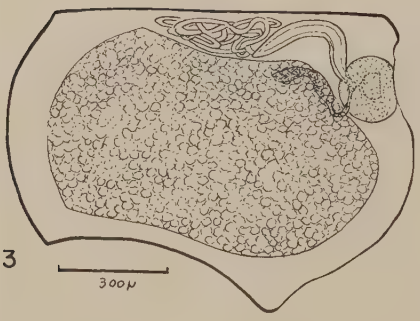

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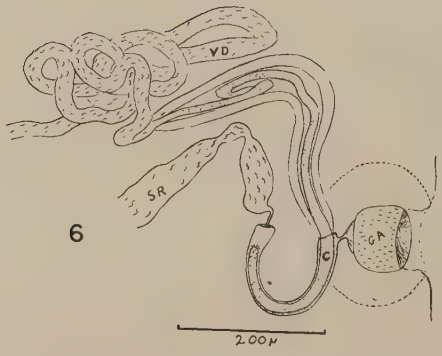

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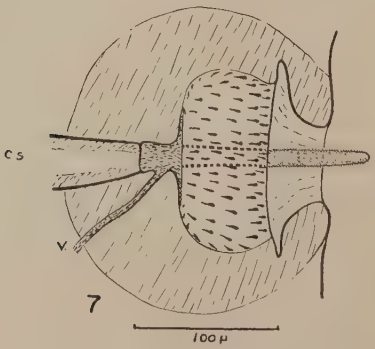

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EFFECTIVENESS OF PIPERONYL BUTOXIDE AND PYRETHRUM AS A PRACTICAL TREATMENT FOR HOG LICE

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The hog louse, *Haematopinus suis* (L.), is of widespread occurrence on hogs and it is said to be, next to cholera, the worst enemy of swine. It is the largest blood-sucking louse found on any farm animal. It causes needless irritation and loss of blood and makes the animals restless so that they fail to gain in weight in a normal manner. The habits of hogs in sleeping and eating together enhances the rapid spread of lice from one animal to another until the entire herd is soon infested.

Application of a thin even coat of oil over the whole body of the hog, either by brushing or by dipping has been the usual measure recommended for control of hog lice. Several kinds of oils, especially crude petroleum, raw linseed oil, equal parts of kerosene and lard, equal parts of kerosene and cottonseed oil, and processed fuel oil, have been recommended as treatments. Also coal tar-creosote dips and pine tar dips have been used for control of hog lice. When oil treatments are used, however, it is necessary to treat every animal present and to repeat the application in two weeks in order to kill the newly hatched lice before they are capable of laying eggs. Following this treatment, the hogs should be kept out of the sunlight and not driven or excited, for at least a day after oiling.

Results of certain phases of extensive studies on the human louse have furnished basic information which has been applied to the control of hog lice. The human body louse, *Pediculus humanus corporis* Deg., is considered to be highly resistant to insecticides. Finely ground pyrethrum flowers and derris powder were found by Bushland, McAlister, Eddy, Jones, and Knipling (1944), to be comparatively ineffective, but powders prepared by depositing pyrethrum extract on an inert diluent were highly effective. Certain synergists used with powders impregnated with pyrethrum extract increased the effectiveness of pyrethrins against body lice approximately 100 times. Impregnation of underwear with pyrethrum extract and a suitable synergist with as little as 0.28 gm of pyrethrins per suit has been shown by Jones, McAlister, Bushland, and Knipling (1944) to be effective against nymphs and adults of the body louse introduced after the garments had been worn for six weeks following treatment. Subsequent to this work, piperonyl butoxide has been found to increase the effectiveness of pyrethrins against body lice to a much greater extent than that indicated for the synergist used in war-developed pyrethrum louse treatments.

Dove (1947) has shown that piperonyl butoxide is an effective insecticide against houseflies, and that its effectiveness is greatly increased by the incorporation of a small proportion of pyrethrins. The structural formula of piperonyl butoxide, 3,4-methylenedioxy-6-propylbenzyl butyl diethylene glycol ether, has been described by Wachs (1947). Fortunately this material makes it possible to employ pyrethrins in very low concentrations and to obtain effectiveness which would be uneconomical, even if feasible, were pyrethrins used alone. The addition of piperonyl butoxide to a pyrethrum insecticide formulation does not introduce toxicological hazards.

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LABORATORY TESTS AGAINST HOG LICE

A series of laboratory tests was conducted to determine the effectiveness against the hog louse of technical piperonyl butoxide[1] combined with a low proportion of pyrethrins in hog oil, which is described as an oil derived from a crude oil with paraffin character. It was supplied by a commercial oil company, has an unsulfonated residue of 84 per cent, 70/80 Saybolt viscosity at 100° F, flash point above 335° F, and NPA color maximum of 2½.

The method used in the laboratory tests with the hog louse was essentially the same as that described under *beaker tests* by Bushland et al (1944). The hog-oil solution containing the different concentrations of technical piperonyl butoxide and pyrethrins was sprayed on pieces of O.D. woolen cloth at the rate of 5 ml per square

TABLE 1.—*Effectiveness against hog lice in laboratory tests of technical piperonyl butoxide and pyrethrins in hog oil applied on O.D. woolen cloth. Baltimore, Maryland, 1947*

Treatment	Deposit per square foot		Percentage survival of lice 24 hours after exposure on cloth sprayed indicated days earlier					
	Technical piperonyl butoxide	Pyrethrins	1 day	3 days	4 days	5 days	7 days	11 days
	Milligrams	*Milligrams*						
2 gm piperonyl butoxide per 100 ml in hog oil ..	100	..	0	70	100	70	100	...
2 gm piperonyl butoxide and 0.05 gm pyrethrins per 100 ml in hog oil ..	100	2.5	0	0	20	30	30	100
0.5 gm piperonyl butoxide and 0.05 gm pyrethrins per 100 ml in hog oil ..	20	2.5	0	0	30	30	10	100
1 gm piperonyl butoxide and 0.1 gm pyrethrins per 100 ml in hog oil ..	50	5	10	0	0	30	0	100
Hog oil (alone)	...	..	10	80	100	100	70	100
White mineral oil* (alone)	...	..	...	100	100	100	80	100
None	...	..	100	100	100	100	100	100

* A water white mineral oil of 50/55 Saybolt viscosity at 100° F and flash point of 295/300° F.

foot. When the oil film had dried, circular pieces of the cloth were cut to fit the bottom of a 50-ml beaker, and 10 adult hog lice were introduced. The lice were exposed on the treated cloth for only 15 minutes, after which they were removed to an untreated piece of cloth in a similar clean beaker and held in an incubator (temperature of 30° ± 2° C and humidity approximately 60 per cent) to determine the mortality at the end of 24 hours. Lice were exposed on the treated pieces of cloth on the first, the third, the fourth, and fifth, the seventh, and the eleventh day after spraying.

The results obtained in the laboratory tests with the hog oil alone, a white mineral oil alone, technical piperonyl butoxide alone in the hog oil, and technical piperonyl butoxide and pyrethrins in the hog oil are shown in table 1.

The results of the laboratory tests against hog lice indicated that the hog-oil solution containing 0.5 gm of technical piperonyl butoxide and 0.05 gm of pyrethrins per 100 ml applied at the rate of 5 ml per square foot killed in 24 hours all of the lice exposed for only 15 minutes. On treated pieces of cloth, this concentration

[1] Technical piperonyl butoxide contains not less than 80 per cent of 3,4-methylenedioxy-6-propylbenzyl butyl diethylene glycol ether and not more than 20 per cent of other closely related compounds.

at the dosage used was effective through the third day after spraying. The hog-oil solution containing 1 gm of technical piperonyl butoxide and 0.1 gm of pyrethrins per 100 ml was effective through the fourth day after application, and that containing 2 gm of technical piperonyl butoxide and 0.05 gm of pyrethrins per 100 ml was effective through the third day only. The hog oil containing 2 gm of technical piperonyl butoxide per 100 ml was not significantly more effective than the hog oil alone which permitted lice to survive even on the first day. A white mineral oil of 50/55 Saybolt viscosity at 100° F was also used, and it was not as effective as the hog oil. Technical piperonyl butoxide and pyrethrins were not tested in the white mineral oil.

BARNYARD TESTS AGAINST HOG LICE

The lowest concentration, 0.5 gm of technical piperonyl butoxide and 0.05 gm of pyrethrins per 100 ml in the hog oil which had given effective results in the laboratory was also tested on hogs in several barnyards against heavy infestations of hog lice. Five shoats weighing about 30 pounds each, all of which were heavily infested with lice, were sprayed with one-fourth ounce of the hog-oil solution per animal by directing the stream down the middle of the back. The shoats were examined thoroughly every other day during the first week, and no living lice could be found. The examination at the end of the second week, however, disclosed a few newly hatched nymphs, but apparently most of these succumbed since only one small nymph could be found at the end of the third week. The shoats were examined again one month after application of the treatment, and no living lice or eggs could be found on them.

Two very large sows were each sprayed with one-fourth ounce of the same material by directing the stream down the middle of the back. At the end of one week, a few lice were still surviving on one of the animals. It was believed that the small quantity of solution used on such large hogs was not sufficient to provide adequate coverage to affect all of the lice. The sows were then sprayed with an additional one-half ounce of the hog-oil solution, and careful examinations made at weekly intervals over a period of one month revealed no living lice or eggs.

Four young hogs were sprayed in the manner previously described with one-fourth ounce of the hog-oil solution per animal. Observations made during the first week after treatment disclosed no lice, but during the second week a few newly hatched nymphs were found on each of the hogs. They were again sprayed with the same quantity of material, and examinations over a four-week period following the second application revealed no lice.

A final test was made on a group of 30 hogs in a corral of about one-fourth acre containing many trees. On examination before treatment, all of the hogs were found to be heavily infested with lice. A post, $4'' \times 4'' \times 6'$, was set firmly in the ground inside the hog lot, and burlap 18 inches wide was wrapped around the post to give four layers and was nailed securely. The hog-oil solution was then sprayed on the burlap at the rate of $3\frac{1}{4}$ ounces (96.1 ml) per square foot. Within a very short time most of the hogs were observed to rub against the newly set post (Fig. 1) and despite the presence of many trees apparently all of the hogs rubbed at least one time against the post. Small patches of oil could be readily seen on the hogs.

The 30 hogs were thoroughly examined the next day, and they were also examined at weekly intervals over a four weeks period. No living lice were found at any of the examinations.

DISCUSSION AND CONCLUSIONS

The Laboratory and barnyard tests show that the hog louse can be effectively controlled by application to each animal of a small quantity of a hog oil containing 0.5 gm of technical piperonyl butoxide and 0.05 gm of pyrethrins per 100 ml. A group of pigs or hogs in a corral could be effectively treated by the use of a rubbing post wrapped with several thicknesses of burlap on which the hog-oil solution is

FIG. 1. Hogs readily rubbed against a post padded with burlap which was sprayed lightly with hog-oil solution of technical piperonyl butoxide and pyrethrins.

sprayed. The burlap should be sprayed every three or four weeks. Automatic mechanical hog-oilers may be used in the same manner. Small groups of swine confined in pens should be sprayed individually with a small quantity of the solution. The first spraying should be followed by a second application in two weeks, after which the animals can be kept free of lice by spraying lightly about once a month.

This type of treatment makes unnecessary the application of a film of oil over the whole body of the hog, thereby reducing the danger of exposing oil-treated animals to the sunlight. Hog lice move around on the animal in much the same manner as do body lice on man. Spot treatments on man were reported by Buxton[2] to be effective against body lice, but the time required by this method to eliminate lice was too long, particularly when the threat of disease transmission was imminent. The hog louse is not known to transmit a disease, hence its elimination in a short

[2] Buxton, P. A. Personal communication.

period is not of prime importance. In moving around over wide areas, the hog lice have frequent opportunity to come in contact with treated spots on the hogs. This makes it possible for an effective insecticide to be easily applied in economical quantities.

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RESEARCH NOTES

THE ANATOMY OF MESOCESTOIDES—CORRECTIONS

The writer (1946, J. Parasitol. **32**: 242–246) described details of the anatomy of the reproductive systems of *Mesocestoides latus*, showing that the egg capsule is a true parauterine organ. The statement was made that this organ had been considered the posterior portion of the uterus by all previous writers. Unfortunately, and inexcusably, a paper by E. E. Byrd and J. W. Ward (1943, J. Parasitol. **29**: 217–226) had completely escaped notice. In this paper the true nature of the egg capsule and the relationships of the various portions of the female reproductive system are fully and correctly described. It also appears, from Byrd and Ward's studies, using sectioned material, that the organ which the writer called an oötype, and which he believed to be surrounded by Mehlis' glands, is in reality an oöcapt, the Mehlis' glands surrounding the uterine duct in the usual position just beyond the entrance of the common vitelline duct, as had previously been illustrated by Fuhrmann. These glands are not in evidence in the *M. latus* material studied by the writer, but might have been demonstrated, as they were by Byrd and Ward in *M. variabilis*, in sections.

Another interesting point is made by Byrd and Ward. They consider that the genital pore is on the dorsal surface, instead of the ventral surface as described by the writer and others. This interpretation is based on the fact that the pore is on the opposite surface from that to which the ovaries are approximated. In this particular instance, as Byrd and Ward point out, the positions of the excretory tubes are of no help, since they both lie in the same dorsoventral plane. If the position of the ovaries is accepted as a criterion for determining the dorsal and ventral surfaces, as it has been in the past, the dorsal position of the genital pore must be accepted. In view of this, the terms dorsal and ventral, and dorsad and ventrad, in the writer's 1946 paper should be reversed; the relative position of the various organs and tubes remains, of course, unchanged. Minor differences in the shape of organs and course of ducts in Bryd and Ward's description of *M. variabilis* and in the writer's of *M. latus*, may prove to be useful distinguishing characteristics between these two species.—Asa C. Chandler, *Rice Institute, Houston, Tex.*

A NEW HOST FOR *CLINOSTOMUM* METACERCARIAE

In the spring of 1941 an adult spotted salamander, *Ambystoma maculatum* (Shaw), was collected along the Potomac River above Seneca, Montgomery County, Maryland. This salamander was kept in captivity until October 3, 1941, at which time numerous white swellings were noted on its head and body. These swellings proved to be dermal cysts, each one of which contained a fluke metacercaria. Specimens of these parasites were identified by Mr. Allen McIntosh of the Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland, as the metacercariae of *Clinostomum* sp., the adults of which are parasites of herons and other birds of similar habits.

Metacercarial stages of clinostomes have previously been reported from fishes and frogs and also from a West Indian land snail, *Subulina octona* (McIntosh, 1935, Proc. Helminth. Soc. Wash. **2**(2): 79–80). They have also been recorded from two other salamanders. Bennett and Humes (1938, Proc. La. Acad. Sci. **IV**(1): 243–245) have recorded them from *Amphiuma tridactylum* Cuvier in Louisiana, and Manter (1938, Trans. Amer. Micr. Soc. **57**(1): 26–37) has recorded them from *Siren lacertina* Linné in Florida.

The occurrence of these metacercariae in *Ambystoma maculatum* adds another salamander to the list of cold-blooded animals serving as hosts for the metacercarial stages of *Clinostomum*. It is probable that this particular specimen of *A. maculatum* owed its infection to a colony of yellow-crowned night herons that were nesting nearby. Thus while this salamander is essentially terrestrial, its skin was perhaps penetrated by the cercariae of this fluke while it was in the water during the spring breeding season. In this connection it is interesting to note that both *A. tridactylum* and *S. lacertina* are completely aquatic salamanders.—J. A. Fowler, *The Academy of Natural Sciences, Philadelphia, Pa.*

ON THE MITE *CHEYLETIELLA PARASITIVORAX*, OCCURRING ON CATS, AS A FACULTATIVE PARASITE OF MAN

Cooper (1946, J. Parasitol. **32**: 480–482) recently gave a survey of the literature on *Cheyletiella parasitivorax* (Mégnin, 1878), syn. *Ewingella americana* Vail & Augustson, 1943. While this mite (family Cheyletidae) had originally been found only in the fur of rabbits and hares, where it was said to feed on other mites, such as listrophorids, it is now known also to

occur on the hairs of cats (Denmark, England, USA), possibly sometimes feeding on the cat mange mite, *Notoedres cati.*

Lomholt, Jersild and Boas, at the meeting of May 2, 1917 of the Danish Dermatological Society (1918, Hospitalstidende **61:** 1098–1099), reported occurrences of dermatitis in two families each of which had a cat heavily infested with *C. parasitivorax.* In spite of these observations and other authors' finds of skin lesions in rabbits harboring these mites, Cooper did not consider it sufficiently established that they should be annoying to their mammalian hosts or even man. Kuscher (1940, Wien. Tierärztl. Monatsschr. **27:** 10–16), however, in a paper not quoted by Cooper, described *C. parasitivorax* from a dog, where it was associated with the follicular mite *Demodex canis.* The round skin lesions caused by the latter were surrounded by red, somewhat elevated, almost hairless "randzones," covered by a layer of heaped-up, furfuraceous scales, among which *C. parasitivorax* were abundant. According to Kuscher, the latter mites which originally had been feeding on the *Demodex,* thereby being beneficious to the host, were also able to produce those skin lesions in the "randzones" where the follicular mites had already been exterminated.

Quite recently, two new instances were observed in Denmark of human dermatitis, traceable to domestic cats, infested with *C. parasitivorax.* In one of these cases, which was reported by Lomholt (1947, Ugeskr. f. Læger **109:** 228–229), all three members of a family had throughout a year to a somewhat varying degree been suffering from an heavily itching, papulous eczema, which had several times in vain been treated as scabies. Finally the cause was detected in the domestic cat that harbored a great number of *Cheyletiella.* The cat was destroyed, and all the affected persons recovered.

In the other case, seen by us, a Copenhagen family that had owned a healthy angora cat for two years acquired another angora cat. The new cat was often seen scratching itself, and shortly after also the old angora cat and its five kittens began scratching themselves, while the two owners became affected with a rather mysterious papulous, intensely itching eczema which withstood all kind of treatment. The eruptions were worse whenever the cats had been in the beds. On the other hand, the condition improved in the course of from 8 to 14 days, when the owners left their home for a journey (Lomholt made the same observation in his case). Altogether nine persons who for a shorter or longer while came in a somewhat intimate contact with the cats presented symptoms. An examination of the animals proved them to be infested with *C. parasitivorax* in the fur as well as with the ear mite, *Otodectes cynotis,* in the ears. After six of the cats had been treated with a derris solution and the seventh with DDT powder, no more mites could be traced on them, their scratching subsided and their owners soon let them sleep in their beds again, without getting new eruptions.

From the histories here reported it seems obvious that under certain circumstances *C. parasitivorax* may become noxious to its mammalian host and that at times it even attacks human beings where it is the cause of a very annoying eczema. In a preliminary experiment, where two mites were placed on the forearm, one of them stood still twice for a few minutes with its fore-end bent down to the skin and there produced two red, slightly elevated spots. These reactions, however, were considerably weaker than those seen in the patients, the difference being possibly due to the fact that the patients had mostly been attacked in the warmth of the bed and in the peace and darkness of the night.—Sven J. Olsen and Hans Roth, *Hygienic-Bacteriological Department (Chief: Prof. Aa. Jepsen), The Royal Veterinary and Agricultural College, Copenhagen, Denmark.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Preliminary Announcement of the Twenty-Second Annual Meeting

Monday, Tuesday, and Wednesday, December 29 to 31, 1947
Chicago, Illinois

Following the decision of the Council and action taken by the Society at the Boston (1946) meeting, the American Society of Parasitologists will convene for a three-day meeting in conjunction with the convention of the American Association for the Advancement of Science in Chicago, Illinois. The Congress Hotel will be the official headquarters for the Society and all functions will be held at that Hotel.

Sessions for contributed papers, to be read in person are scheduled principally for the first and third days. Certain features of the program will be arranged for the second day. At the end of the second morning session, Dr. Harley J. Van Cleave will deliver the Presidential Address, which is entitled: "Expanding Horizons in the Recognition of a Phylum." The annual Society Luncheon will be held at noon on the second day, and will be followed by the annual Business Meeting. During the second afternoon, a demonstration program and tea will be arranged. During the afternoon of the third day a symposium of invited papers on the exoerythrocytic forms of the malaria parasites has been arranged by Dr. C. G. Huff, Vice-President of the Society.

All members are urged to attend the convention and to participate actively in the Society functions.

Respectfully,
James T. Culbertson, *Secretary*

Proposed Amendment to the Constitution

The Science Press Printing Co. has announced that, beginning January 1, 1948, printing costs for the Journal of Parasitology will be further increased by 20 per cent. Because other operational expenses of the Society are also greater, the Council has voted to recommend to the annual business meeting that the Constitution of the Society be amended and that the annual dues be increased to $5.00 per year for active members. This announcement provides the 30 days' notice to members of the proposed amendment as required by the Constitution.

APPLICATION FOR HOUSING ACCOMMODATIONS, FOR ANNUAL MEETING, CHICAGO, ILLINOIS, DECEMBER 29TH TO 31ST, 1947

All reservations for hotel rooms or other housing accommodations must be filed not later than November 26, 1947 with

AAAS Reservation Center
Chicago Convention Bureau,
33 North LaSalle Street,
Chicago 2, Illinois.

Name three (3) hotels in order of preference and state the exact date and hour of arrival and departure. Describe precisely what space is needed and give the name of each person who will occupy the quarters. The names and rates for single rooms in representative hotels follow:

Congress Hotel—$4.00 to $10.00
LaSalle Hotel—$3.30 to $6.50
Palmer House—$4.00 to $9.00
Sherman Hotel—$3.95 to $8.95
Stevens Hotel—$4.00 to $9.00

All Society functions are being held in the Congress Hotel.

"MEDICHROME" SERIES MT

Reg. U. S. Pat. Off.

Tropical Diseases

A series of approximately 150 2 x 2" (35 mm.) Kodachrome transparencies (lantern slides), made with the cooperation of DR. HENRY E. MELENEY, DR. HARRY MOST and DR. DOMINIC DeGIUSTI, Department of Preventive Medicine, New York University College of Medicine, Slides Nos. MT45, 46 and 47 are from the Army Medical Museum, Washington, D. C.

VIRUS DISEASES

MT 1. Yellow fever—Liver lobule showing midzonal necrosis H&E, 45X.

BACTERIAL DISEASES

MT 5. Plague—Lung, pneumonic plague 100X.
MT 6. Plague—exudate in alveoli H&E 500X.
MT 7. Plague—section of flea showing plug of bacilli in proventriculus and stomach 100X.
MT 8. Plague bacilli in smear of spleen 540X.
MT 10. Bacillary dysentery—smear of exudate in stool. Iron Htx 320X.
MT 127. Bacillary dysentery, section of colon H&E 10X.
MT 127.1. Bacillary dysentery, section of colon showing type of inflammatory reaction H&E 65X.
MT 15. Granuloma inguinale—donovan bodies in macrophage—capsulated-giemsa 540X.

RICKETTSIAL DISEASES

MT 21. Smear of rickettsiae—giemsa 720X.
MT 22. Section of blood vessel showing thrombus 15X.

SPIROCHETAL DISEASES

MT 25. Relapsing fever—spirochaetes smear of blood—giemsa 720X.
MT 26. Rat-bite fever—spirilla, smear of blood—giemsa 1000X.
MT 27. Leptospirosis—kidney of rat—leptospira—silver nitrate 550X.
MT 28. Leptospirosis—liver of guinea pig—leptospira—silver nitrate 1000X.

FUNGUS DISEASES

MT 30. Histoplasmosis—section of lung, liver, or spleen showing Histoplasma in macrophages H&E 630X.
MT 30A. Histoplasmosis—smear of blood 720X.
MT 35A. Blastomycosis—section of lung showing Blastomyces H&E 160X.
MT 35. Blastomycosis—section of lung showing Blastomyces H&E 720X.
MT 36A. Coccidioidomycosis — Coccidioides immitis, section of tissue H&E 1.p.
MT 36. Coccidioidomycosis — Coccidioides immitis, section of tissue H&E 1000X.
MT 40. Rhinosporidium seeberi, nasal polyp 65X.
MT 38. Actinomycosis—section of brain showing rays H&E 1000X.
MT 39. Actinomycosis—section of lung showing rays H&E 1000X.

PROTOZOAN DISEASES

MALARIA

MT 45. Plasmodium vivax, life history from drawings.
MT 46. Plasmodium malariae, life history from drawings.
MT 47. Plasmodium falciparum, life history from drawings.
MT 55. Smear showing engulfed malarial pigment in w.b.c. giemsa 720X.
MT 56. Plasmodium falciparum, smear of brain, capillaries, l.s. H&E 500X.
MT 56A. Plasmodium falciparum, smear of brain, capillaries, l.s. H&E 1200X.
MT 56B. Plasmodium falciparum, section of brain, capillaries, c.s. 1200X.
MT 57. Section of liver showing malarial pigment without parasites 300X.
MT 58. Mosquito stomach showing oocysts of P. vivax 40X.
MT 60. Malaria—sporozoites, giemsa 720X.
MT 61. Malaria—exflagellation of male gametocyte 1250X.

TRYPANOSOMIASIS

MT 69. African trypanosomiasis — Trypanosoma brucei, smear of fat blood, giemsa 1000X.
MT 69A. African trypanosomiasis—Trypanosoma gambiense, smear of human blood, giemsa 1250X.
MT 65. African trypanosomiasis—section of brain showing perivascular infiltration H&E 40X.
MT 78. Trypanosoma lewisi—smear of rat blood, giemsa 630X.
MT 80A. South American trypanosomiasis (Chagas disease)—Trypanosoma cruzi, section of heart muscle showing leishmaniform stage H&E 500X.
MT 80. Trypanosoma cruzi—smear of culture, showing chrithidia form, giemsa 1000X.

LEISHMANIASIS

MT 88A. Leishmania donovani (Kala Azar)—smear of human blood giemsa 720X.
MT 88. Leishmania donovani—smear of human bone marrow giemsa 720X.
MT 85. Leishmania donovani—section of liver H&E 720X.
MT 92A. Leishmania donovani—tropica 720X.

INTESTINAL FLAGELLATES

MT 123. Giardia lamblia, motile, smear of feces, Iron Htx 720X.
MT 96. Giardia lamblia, cysts, smear of feces, Iron Htx 720X.
MT 120. Chilomastix mesnili, motile, smear of feces, Iron Htx 720X.
MT 97. Trichomonas hominis, motile, giemsa stain 720X.

INTESTINAL CILIATES

MT 125. Balantidium coli, motile, 500X.
MT 126. Balantidium coli, motile, section of ulcer of colon, H&E 100X.

INTESTINAL AMEBAE

Amebiasis—E. Histolytica

MT 109. E. Histolytica, motile, smear of feces, Iron Htx & Eosin 1000X.
MT 100. E. histolytica, ulcer of intestine, H&E 100X.
MT 101. E. histolytica, ulcer of intestine, H&E 500X.
MT 103. E. histolytica, ulcer of intestine, H&E 1000X.
MT 105. E. histolytica, abscess of liver, H&E 45X.
MT 105A. E. histolytica, abscess of liver, H&E 250X.
MT 107. E. histolytica, ulcer of skin, H&E 720X.
MT 107.1. E. histolytica, amebae invading skin, H&E 100X.
MT 107B. E. histolytica, amebae invading fat (skin) H&E 100X.
MT 107A. E. histolytica, amebae in lung, H&E 60X.
MT 107.2. E. histolytica, amebae in lung, H&E 300X.

Amebae, other than E. histolytica

MT 111. E. coli, motile, smear of feces, Iron Htx 720X.
MT 114. Endolimax nana, motile, smear of feces, Iron Htx 720X.
MT 116. Iodameba butschlii, motile, smear of feces, Iron Htx 720X.

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